

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number  
WO 02/29022 A2

(51) International Patent Classification<sup>7</sup>: C12N 9/18, 15/63, 15/82, A01H 5/10

(21) International Application Number: PCT/US01/31059

(22) International Filing Date: 4 October 2001 (04.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/238,161 5 October 2000 (05.10.2000) US

(71) Applicant (for all designated States except US): E. I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(74) Agent: GOLIAN, Paul, D.; E. I. DU PONT DE NEMOURS AND COMPANY, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CAHOON, Edgar, B. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US). CAHOON, Rebecca, E. [US/US]; 2331 West 18th Street, Wilmington, DE 19806 (US). THORPE, Catherine, J. [GB/GB]; 49 Beech Road, St. Albans, Hertfordshire AL3 5AP (GB).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CHLOROPHYLLASES

SEQ ID NO:19 MAAIIDESPTFSS---VVT PAA-----FEIGSLPTTEIPVDPV-----ENDST  
SEQ ID NO:20 MS-SSSSR-----NAFDGKYKSNLITLD---SSS RCKITPSSRASP  
SEQ ID NO:22 MAAMVDAKPAAS---VQGTPLLA---TATLPVFTRGIYSTKRITLETSSPS---SP  
SEQ ID NO:21 MAKLLL LIEGVFIFVNSQAQTFPTILEKHNS EKITDVFHKGNFQVTNNPI-----RVKRYEF  
SEQ ID NO: 2 -----RVET-----Sニア-  
SEQ ID NO: 4 STCSTAA-----NVFEIGKHITVLLRAE---PGT--C--TTKSSLPV  
SEQ ID NO: 6 MNLASAVRFLSYYLLVQRWMGS---EQGGGVFDQGGHSVSLTRLDEARAPPRCAVRSSPSSAAS  
SEQ ID NO: 8 MAQR AEPILVT-----TDVFQMGNIKWKQFNIDT-----SNASS  
SEQ ID NO:10 MAQR AQPVLAT-----DVFQMGNIQWKQFNVDT-----SSASF  
SEQ ID NO:12 MAQR AQPALAT-----TDVFQKGDIHWKQFNVET-----STASS  
SEQ ID NO:14 MASSPCS-----VFPVPGKYTVQLKSVE--AGTK--KARHVSSVA  
SEQ ID NO:16 -----  
SEQ ID NO:18 MAAAAPAETMNK---SAAGAEVP---EAFTSVFQPGKL AVEAIQVDE-----NAAP  
SEQ ID NO:26 MAAMA-----TTVFQAGPMEVDVKHVDK-----SMIP

1

70

(57) Abstract: This invention relates to isolated polynucleotides encoding chlorophyllases. The invention also relates to the construction of recombinant DNA fragments comprising all or a portion of the isolated chlorophyllase-encoding polynucleotides, in sense or antisense orientation, wherein expression of the recombinant DNA fragment results in production of altered levels of chlorophyllase in a transformed host cell.

WO 02/29022 A2

TITLE  
CHLOROPHYLLASES

This application claims the benefit of U.S. Provisional Application No. 60/238,161 filed October 05, 2000.

5 FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding chlorophyllases in plants and seeds.

BACKGROUND OF THE INVENTION

10 Fruits, stems, and leaves change color in response to developmental and environmental factors. Although these tissues have very different functional, morphological, and biochemical attributes their cells undergo similar changes in ultrastructure and chloroplast composition during fruit ripening and leaf senescence. Chlorophyll breakdown is a primary biochemical event in color changes and the first 15 step in the chlorophyll degradation pathway is catalyzed by chlorophyllase (EC 3.1.1.14). This enzyme catalyzes the hydrolysis of the phytol chain in chlorophylls or pheophytins to produce chlorophyllides or pheophorbides. Chlorophyllases of different mobilities on polyacrylamide gels have been purified from plants and algae and in some instances, like in citrus, there are two 20 chlorophyllase bands present. It is not known what the difference in size represents.

Genes encoding chlorophyllases have been purified from Valencia oranges and *Chenopodium album*. The Valencia orange Chlase1 gene was isolated from a library prepared from mRNA extracted from the fruit peel, stems and leaves. The 25 steady state level of Chlase1 mRNA increased with ethylene treatment (Jacob-Wilk, et al. *Plant J.* (1999) 20:653-661). Although three proteins, of different molecular weight, with chlorophyllase activity have been isolated from *Chenopodium album*, only the gene encoding one of them (CaCLH C. *alum* chlorophyll-chlorophyllido hydrolase) has been identified (Tsuchiya et al. (1999) *Proc. Natl. Acad. Sci. USA* 30 96:15362-15367). Using sequence similarity to the C. *album* CaCLH chlorophyllase sequence two *Arabidopsis thaliana* genes have also been assigned chlorophyllase activity. Tsuchiya et al. refer to these *Arabidopsis* genes as AtCHL1 and AtCHL2, respectively. Expression of CaCLH, AtCHL1, and AtCHL2 in *E. coli* resulted in chlorophyllase activity (Tsuchiya et al., *supra*).

35 The CaCLH deduced amino acid sequence has between 32% and 40% sequence homology with that of AtCHL1 and AtCHL2. All three polypeptides have highly conserved regions that correspond to a conserved motif present in several

bacterial, animal, and plant lipases and hydrolases which includes a potential ATP/GTP-binding-site motif, or P-loop. Homology of the entire polypeptide of any of the chlorophyllases with other known lipases is less than 10%.

AtCHL2 was originally labeled COI1 and was isolated from an *Arabidopsis thaliana* mutant (*coi1*, for coronatine-insensitive 1) which is insensitive to methyl jasmonate (MeJA) and coronatine and produces sterile male flowers. AtCHL1 was originally labeled ATHCOR1 (for *Arabidopsis thaliana* coronatine induced) and was identified as being induced by MeJA, coronatine, and wounding (Benedetti et al. (1998) *Plant Physiol.* 116:1037-1042). Coronatine is a phytotoxin produced by some plant-pathogenic bacteria, and mimics the action of MeJA in plants. MeJA is a plant-signaling molecule involved in stress responses such as wounding and pathogen attack and is essential for pollen grain development in *Arabidopsis thaliana*.

Because of the involvement of chlorophyll degradation in plant cell senescence, identification of cDNAs encoding chlorophyllases in other plants, particularly economically important crop plants, will allow the controlled induction or postponement of senescence.

#### SUMMARY OF THE INVENTION

The present invention concerns isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide having chlorophyllase activity wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 have at least 80% sequence identity. It is preferred that the identity be at least 85%, it is preferable if the identity is at least 90%, it is more preferred that the identity be at least 95%. The present invention also relates to isolated polynucleotides comprising the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary. More specifically, the present invention concerns isolated polynucleotides encoding the polypeptide sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 or nucleotide sequences comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25.

In a second embodiment, this invention relates to a vector comprising the polynucleotide of the present invention or a recombinant DNA construct comprising the polynucleotide of the present invention operably linked to at least one regulatory sequence.

In a third embodiment, the invention concerns a cell comprising the recombinant DNA construct of the present invention. The cell may be a eukaryotic cell such as a plant cell, or a prokaryotic cell such as a bacterial cell.

5 In a fourth embodiment, the invention relates to a method of transforming a cell by introducing into the cell a nucleic acid comprising a polynucleotide of the present invention. The invention also concerns a method for producing a plant comprising transforming a plant cell with a nucleic acid molecule comprising a polynucleotide of the present invention and regenerating a plant from the transformed plant cell. In a further embodiment, the seed from the transformed plant is included.

10 In a fifth embodiment the invention concerns a method for isolating a polypeptide encoded by the polynucleotide of the present invention comprising isolating the polypeptide from a cell transformed with a nucleic acid comprising said polynucleotide.

15 In a sixth embodiment the invention relates to an isolated chlorophyllase polypeptide comprising an amino acid sequence having a sequence identity of at least 80% when compared to an amino acid sequence having an amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26. It is preferred that the identity be at least 85%, it is more preferred if the identity is at least 90%, it is preferable that the identity be at least 95%. The invention is related to a polypeptide of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26.

20 BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

25 Figures 1A-1F show a comparison of the amino acid sequences of the grape clone vdb1c.pk002.p19:fis (SEQ ID NO:2), grape clone vrl1c.pk008.o21:fis (SEQ ID NO:4), corn clone csh3c.pk001.a9:fis (SEQ ID NO:6), soybean clone sfl1.pk0046.f8 (SEQ ID NO:8), soybean clone sfl1n1.pk002.m10:fis (SEQ ID NO:10), soybean clone sl2.pk130.f15:fis (SEQ ID NO:12), tulip clone etp1c.pk005.d16:fis (SEQ ID NO:14), wheat clone wl1.pk0012.d7:fis (SEQ ID NO:16), wheat clone wle1n.pk0058.a4:fis (SEQ ID NO:18), wheat clone wyr1c.pk005.f22:fis (SEQ ID NO:26), and the chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier Nos. 2460203 and 6729677, SEQ ID NO:19 and SEQ ID NO:20, respectively), *Chenopodium album* (NCBI General Identifier No. 6729675, SEQ ID NO:21), and *Citrus sinensis* (NCBI General Identifier No. 7328567, SEQ ID NO:22). Amino acids conserved among all the sequences are indicated by an asterisk (\*) above the alignment. The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine

(from the same article) is written in white and boxed in black. Dashes are used by the program to maximize the alignment. Figure 1A, amino acids 1 through 70; Figure 1B, amino acids 71 through 140; Figure 1C, amino acids 141 through 210; Figure 1D, amino acids 211-280, Figure 1E, amino acids 281 through 350; Figure 1F, amino acids 351 through 397.

Figures 2A-2B show a comparison of the amino acid sequences of the corn clone csc1c.pk006.l9:fis (SEQ ID NO:28), and the chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier Nos. 2460203 and 6729677, SEQ ID NO:19 and SEQ ID NO:20, respectively), *Chenopodium album* (NCBI General Identifier No. 6729675, SEQ ID NO:21), and *Citrus sinensis* (NCBI General Identifier No. 7328567, SEQ ID NO:22). Amino acids conserved among all the sequences are indicated by an asterisk (\*) above the alignment. The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine (from the same article) is written in white and boxed in black. Dashes are used by the program to maximize the alignment. Figure 2A, positions 1 through 210; Figure 2B, positions 211 through 384.

Table 1 lists the chlorophyllase polypeptides that are described herein, the plant species from which the polypeptide is derived, the designation of the cDNA clones that comprise the nucleic acid fragments encoding all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

25

TABLE 1  
Chlorophyllases

Species	Clone Designation	SEQ ID NO: (Nucleotide)	SEQ ID NO: (Amino Acid)
Grape	vdb1c.pk002.p19:fis	1	2
Grape	vrl1c.pk008.o21:fis	3	4
Corn	csh3c.pk001.a9:fis	5	6
Soybean	sfl1.pk0046.f8	7	8
Soybean	sfl1n1.pk002.m10:fis	9	10
Soybean	sl2.pk130.f15:fis	11	12
Tulip	etp1c.pk005.d16:fis	13	14
Wheat	wl1.pk0012.d7:fis	15	16
Wheat	wle1n.pk0058.a4:fis	17	18
<i>A. thaliana</i> COI1	GI 2460203		19

Species	Clone Designation	SEQ ID NO: (Nucleotide)	(Amino Acid)
<i>A. thaliana</i> CLH2	GI 6729677	20	
<i>C. album</i> CLH	GI 6729675	21	
<i>C. sinensis</i>	GI 7328567	22	
Oligonucleotide Primer	Primer P1	23	
Oligonucleotide Primer	Primer P2	24	
Wheat	wyr1c.pk005.f22:fis	25	26
Corn	csc1c.pk006.l9:fis	27	28
Oligonucleotide Primer	Primer P3	29	
Oligonucleotide Primer	Primer P4	30	

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) 5 and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The 10 terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A 15 polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, or 20 25 or the complement of such sequences.

The term "isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also 25

embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid

fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art.

5 Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for 10 another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity 15 of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic 20 Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes 25 determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent 30 conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms 35 commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid

sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences 5 reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at 10 least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the 15 default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification 20 of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the 25 explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a 30 known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more 35 nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford

specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now 5 use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

"Codon degeneracy" refers to divergence in the genetic code permitting 10 variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide 15 codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

"Synthetic nucleic acid fragments" can be assembled from oligonucleotide 20 building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to a nucleic acid fragment, means that the component nucleotides were assembled 25 *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. 30 The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, 35 including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene

that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but 5 arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign-gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" 10 is a gene that has been introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA 15 processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence 20 is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a 25 promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to 30 different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further 35 recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

"3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into polypeptides by the cell. "cDNA" refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. "Sense-RNA" refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term "operably linked" refers to the association of two or more nucleic acid fragments on a single polynucleotide so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding

sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid

5 fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

10 "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. Each protein or polypeptide has a unique function.

"Altered levels" or "altered expression" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

20 "Mature protein" or the term "mature" when used in describing a protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

25 A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include

5 *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, capable of

10 introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., *Cloning Vectors: A*

15 *Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable

20 marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

25 Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

30 "PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

35 Nucleic acid fragments encoding at least a portion of several chlorophyllases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using

sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, 5 ligase chain reaction).

For example, genes encoding other chlorophyllases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific 10 oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In 15 addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

20 In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid 25 fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to 30 generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems, specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. 35 (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide

sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the recombinant DNA fragments of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of chlorophyll in those cells. Manipulation of chlorophyllase activity may be useful for the controlled induction of senescence in agronomic or agricultural applications. For example, expressing a chlorophyllase gene under the control of an inducible promoter will allow plants to mature and age earlier in nurseries, or to mature and age later for their use in florist arrangements or during shipping to retail. Overexpression of chlorophyllase genes in oil-producing crops will result in seeds and extracted oils with reduced chlorophyll-derived pigmentation. Chlorophyllase genes may also be useful to develop male sterility in wheat. It may also be possible to use the chlorophyllase expression as a transformation selection marker where non-transformed tissue will be green and transformed tissue will be non-green. Furthermore, chlorophyllases may be prepared in microbial systems, purified, and added to detergents where they will be used to remove grass and other stains resulting from green plant tissues.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to

a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided.

5 The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Vectors may be constructed comprising the instant isolated polynucleotides or recombinant DNA fragments. The choice of vector is dependent upon the method that will be used to transform host cells. The skilled artisan is well aware of 10 the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells containing the foreign polynucleotide. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), 15 and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis, among others.

For some applications it may be useful to direct the instant polypeptides to 20 different cellular compartments, or to facilitate their secretion from the cell. It is thus envisioned that the recombinant DNA fragment described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding 25 endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

30 It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense 35 RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into

plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant chlorophyllase polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that

direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a recombinant DNA fragment for production of the instant polypeptides. This recombinant DNA fragment could then be introduced into appropriate microorganisms via transformation to provide high 5 level expression of the encoded chlorophyllase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided. (Example 6).

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they 10 are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic 15 acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of 20 individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic 25 mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near 30 isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

35 Nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of

large clones (several to several hundred KB; see Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant

can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

### EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

15 The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

### EXAMPLE 1

#### Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

20 cDNA libraries representing mRNAs from various grape, corn, soybean, tulip, and wheat tissues were prepared. The characteristics of the libraries are described below.

**TABLE 2**  
cDNA Libraries from Grape, Corn, Soybean, Tulip, and Wheat

Library	Tissue	Clone
vdb1c	Grape Developing Bud	vdb1c.pk002.p19:fis
vr1c	Grape Resistant Leaves	vr1c.pk008.o21:fis
csh3c	Corn Shoots and Roots Sprayed With Herbicide*	csh3c.pk001.a9:fis
sfl1	Soybean Immature Flower	sfl1.pk0046.f8
sfl1n1	Soybean Immature Flower**	sfl1n1.pk002.m10:fis
sl2	Soybean Two-Week-Old Developing Seedlings Treated With 2.5 ppm chlorimuron	sl2.pk130.f15:fis
etp1c	Tulip Pistil Developed to 3/4 of its Mature Size	etp1c.pk005.d16:fis
wl1	Wheat Leaf From 7 Day Old Seedling	wl1.pk0012.d7:fis
wle1n	Wheat Leaf From 7 Day Old Etiolated Seedling**	wle1n.pk0058.a4:fis
wyr1c	Wheat yellow rust infested tissue	wyr1c.pk005.f22:fis
csc1c	Corn 20-Day Seedling (Germination Cold Stress). The Seedling Appeared Purple.	csc1c.pk006.l9:fis

- \* Application of *N*-(3,6-dihydro-2*H*-pyran-4-yl)-4-(3,5-dimethyl-4-isoxazolyl)-4,5-dihydro-*N*-(1-methylethyl)-5-oxo-1*H*-tetrazole-1-carboxamide; synthesis and methods of using this compound are described in WO98/35961, incorporated herein by reference.
- 5 \*\*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the 10 cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) 15 vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase 20 chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent 25 sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA templates are reacted with vector primed M13 forward and reverse oligonucleotides 30 in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res.* 22:3765-3772). The *in vitro* transposition system places unique binding sites 35 randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element

contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res.* 11:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via 5 alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred/Phrap (P. Green, University of Washington, Seattle). Phred/Phrap is a public 10 domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (D. Gordon, University of Washington, Seattle).

15 In some of the clones the cDNA fragment corresponds to a portion of the 3'-terminus of the gene and does not cover the entire open reading frame. In order to obtain the upstream information one of two different protocols are used. The first of these methods results in the production of a fragment of DNA containing a portion 20 of the desired gene sequence while the second method results in the production of a fragment containing the entire open reading frame. Both of these methods use two rounds of PCR amplification to obtain fragments from one or more libraries. The libraries some times are chosen based on previous knowledge that the specific 25 gene should be found in a certain tissue and some times are randomly-chosen. Reactions to obtain the same gene may be performed on several libraries in parallel or on a pool of libraries. Library pools are normally prepared using from 3 to 5 different libraries and normalized to a uniform dilution. In the first round of amplification both methods use a vector-specific (forward) primer corresponding to a portion of the vector located at the 5'-terminus of the clone coupled with a gene-specific (reverse) primer. The first method uses a sequence that is 30 complementary to a portion of the already known gene sequence while the second method uses a gene-specific primer complementary to a portion of the 3'-untranslated region (also referred to as UTR). In the second round of amplification a nested set of primers is used for both methods. The resulting DNA 35 fragment is ligated into a pBluescript vector using a commercial kit and following the manufacturer's protocol. This kit is selected from many available from several vendors including Invitrogen (Carlsbad, CA), Promega Biotech (Madison, WI), and

Gibco-BRL (Gaithersburg, MD). The plasmid DNA is isolated by alkaline lysis method and submitted for sequencing and assembly using Phred/Phrap, as above.

### EXAMPLE 2

5

#### Identification of cDNA Clones

cDNA clones encoding chlorophyllases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the genbank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res.* 25:3389-3402) against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 1. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database

using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

5

### EXAMPLE 3

#### Characterization of cDNA Clones Encoding Chlorophyllases

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier No. 2460203 and 6729677), 10 *Chenopodium album* (NCBI General Identifier No. 6729675), and *Citrus sinensis* (NCBI General Identifier No. 7328567). Shown in Table 3 are the BLAST results for the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS") or for the sequences of FISs encoding entire chlorophyllases ("CGS"):

15

**TABLE 3**  
BLAST Results for Sequences Encoding Polypeptides  
Homologous to Chlorophyllase

Clone	Status	2460203	BLAST pLog Score 6729677	6729675	7328567
vdb1c.pk002.p19:fis	FIS	78.22	75.30	64.52	74.70
vrl1c.pk008.o21:fis	FIS	67.15	113.00	49.52	61.52
csh3c.pk001.a9:fis	CGS	54.30	78.40	45.70	53.70
sfl1.pk0046.f8	CGS	78.00	68.40	59.00	na
sfl1n1.pk002.m10:fis	CGS	80.04	66.40	62.10	66.30
sl2.pk130.f15:fis	CGS	76.30	69.30	60.10	66.70
etp1c.pk005.d16:fis	CGS	65.52	87.40	52.10	62.40
wl1.pk0012.d7:fis	FIS	38.30	35.40	35.00	31.00
wle1n.pk0058.a4:fis	CGS	76.00	68.00	55.40	61.04
wyr1c.pk005.f22:fis	CGS	54.40	56.10	51.10	49.00

The nucleotide sequence from the entire cDNA insert in clone 20 vdb1c.pk002.p19:fis is shown in SEQ ID NO:1. Nucleotides 1 through 855 from SEQ ID NO:1 encode the amino acid sequence of SEQ ID NO:2. The nucleotide sequence from the entire cDNA insert in clone vrl1c.pk008.o21:fis is shown in SEQ ID NO:3. Nucleotides 1 through 933 from SEQ ID NO:3 encode the amino acid sequence of SEQ ID NO:4. The nucleotide sequence from the entire cDNA insert in clone csh3c.pk001.a9:fis is shown in SEQ ID NO:5 and it encodes an entire corn chlorophyllase. Nucleotides 71 through 1106 from SEQ ID NO:5 encode the amino

acid sequence of SEQ ID NO:6 with nucleotides 107-109 corresponding to a stop codon. The nucleotide sequence from the entire cDNA insert in clone sf1.pk0046.f8 is shown in SEQ ID NO:7 and it encodes an entire soybean chlorophyllase. Nucleotides 12 through 953 from SEQ ID NO:7 encode the amino acid sequence of SEQ ID NO:8 with nucleotides 954-956 corresponding to a stop codon. The nucleotide sequence from the entire cDNA insert in clone sf1n1.pk002.m10:fis is shown in SEQ ID NO:9 and it encodes an entire soybean chlorophyllase. Nucleotides 26 through 964 from SEQ ID NO:9 encode the amino acid sequence of SEQ ID NO:10 with nucleotides 965-967 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone sl2.pk130.f15:fis is shown in SEQ ID NO:11 and it encodes an entire soybean chlorophyllase. Nucleotides 42 through 989 from SEQ ID NO:11 encode the amino acid sequence of SEQ ID NO:12 with nucleotides 990-992 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone etp1c.pk005.d16:fis is shown in SEQ ID NO:13 and it encodes an entire tulip chlorophyllase. Nucleotides 97 through 1104 from SEQ ID NO:13 encode the amino acid sequence of SEQ ID NO:14 with nucleotides 1105-1107 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wl1.pk0012.d7:fis is shown in SEQ ID NO:15. Nucleotides 1 through 615 from SEQ ID NO:15 encode the amino acid sequence of SEQ ID NO:16 with nucleotides 616-618 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wle1n.pk0058.a4:fis is shown in SEQ ID NO:17 and it encodes an entire wheat chlorophyllase. Nucleotides 47 through 1003 from SEQ ID NO:17 encode the amino acid sequence of SEQ ID NO:18 with nucleotides 1004-1006 corresponding to the stop codon. The nucleotide sequence from the entire cDNA insert in clone wyr1c.pk005.f22:fis is shown in SEQ ID NO:25 and it encodes an entire wheat chlorophyllase. Nucleotides 57 through 1025 from SEQ ID NO:25 encode the amino acid sequence of SEQ ID NO:26 with nucleotides 1026-1028 corresponding to the stop codon.

30 Nucleotides 286 through 669 from soybean clone sf1.pk0046.f8 are 98 % identical to nucleotides 6 through 390 of an EST found in the NCBI database having gi No. 6135059. Nucleotides 24 through 299 from soybean clone sl2.pk130.f15:fis are 100 % identical to nucleotides 1 through 276 of an EST found in the NCBI database having gi No. 7284173. Nucleotides 865 through 1101 from soybean clone sl2.pk130.f15:fis are 100 % identical to nucleotides 18 through 254 of an EST found in the NCBI database having gi No. 6914327. The three EST sequences

mentioned above and found in the NCBI database are identified as having similarities with the coronatine induced protein 1.

Figures 1A-1F present an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26 with the AtCHL1 sequence (SEQ ID NO:19), CaCHL sequence (SEQ ID NO:21), AtCHL2 sequence (SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (SEQ ID NO:22). The lipase and P-loop motif discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) is underlined and the putative active Serine is written in white and boxed in black. As can be seen in these figures, the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26 have a conserved motif comprising (Leu/Ile)-(Ser/Val/Ala)-Gly-His-Ser-(Arg/Lys)-Gly-Gly-(Lys/Gln)-(Thr/Val/Asp)-(Ala/Val)-Phe-(Ala/Ser)-(Leu/Val)-Ala-Leu which includes the putative active Serine.

The data in Table 4 presents the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 26, with the AtCHL1 sequence (NCBI General Identifier No. 2460203, SEQ ID NO:19), the CaCHL sequence (NCBI General Identifier No. 6729675, SEQ ID NO:21), the AtCHL2 sequence (NCBI General Identifier No. 6729677, SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (NCBI General Identifier No. 7328567, SEQ ID NO:22).

20

**TABLE 4**

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Chlorophyllases

Clone	SEQ ID NO.	2460203	6729677	7328567	6729675
vdb1c.pk002.p19:fis	2	49.1	48.4	45.6	43.5
vrl1c.pk008.o21:fis	4	40.5	58.8	36.0	32.5
csh3c.pk001.a9:fis	6	32.7	44.0	32.2	29.5
sfl1.pk0046.f8	8	42.5	38.4	40.0	36.8
sfl1n1.pk002.m10:fis	10	44.7	39.6	39.9	38.0
sl2.pk130.f15:fis	12	43.4	398.9	40.8	36.7
etp1c.pk005.d16:fis	14	39.2	47.5	35.0	33.9
wl1.pk0012.d7:fis	16	36.6	36.6	33.7	38.0
wle1n.pk0058.a4:fis	18	42.0	38.4	37.3	33.9
wyr1c.pk005.f22:fis	26	31.1	34.3	31.0	32.8

25 Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed

using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode one entire corn, three entire soybean, one entire tulip, one entire wheat, and a substantial portion of two grape and one wheat chlorophyllases. These are the first corn, soybean, tulip, wheat, and grape sequences known to Applicant to encode chlorophyllases.

#### EXAMPLE 4

##### Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (Ncol or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes Ncol and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb Ncol-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sall-Ncol promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue®; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase® DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryos borne on suspensor structures proliferates from the scutellum of these immature embryos.

5 The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

10

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

15 The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles 20 (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the 25 supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 µL of ethanol. An aliquot (5 µL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 30 30 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

35 For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a

circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture

5 membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains bialophos (5 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing bialophos. After 6 weeks, areas of

10 about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the bialophos-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After

15 two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

#### EXAMPLE 5

##### Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and

20 transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides

25 downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Ncol (which includes the ATG translation initiation codon), SmaI, KpnI and XbaI. The entire cassette is flanked by HindIII sites.

The cDNA fragment of this gene may be generated by polymerase chain

30 reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

35 Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature

seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, 5 globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

10 Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biostatic® PDS1000/HE instrument (helium retrofit) can be used for these transformations.

15 A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette 20 comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

25 To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second 30 each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

35 Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three

times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

5 Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, 10 clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 6

##### Expression of Chimeric Genes in Microbial Cells

15 The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first 20 destroying the EcoRI and HindIII sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoRI and HindIII sites was inserted at the BamHI site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the NdeI site at the position 25 of translation initiation was converted to an Ncol site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on 30 a 1% low melting agarose gel. Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions, ethanol precipitated, dried and 35 resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs (NEB), Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized.

with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the 5 gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in 10 LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 15 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be 20 observed for protein bands migrating at the expected molecular weight.

#### EXAMPLE 7

##### Determination of Chlorophyllase Activity in Clone wle1n.pk0058.a4

To establish its functional identity, the cDNA insert in clone wle1n.pk0058.a4 was expressed in bacteria and assayed for chlorophyllase activity. Initially, the 25 entire cDNA insert in clone wle1n.pk0058.a4 was amplified by PCR using *Pfu* polymerase (Stratagene) and the oligonucleotide primers P1 (sense, SEQ ID NO:23) and P2 (antisense, SEQ ID NO:24):

P1: 5'-TTTCATATGGCAGCAGCAGCACCCG-3' (SEQ ID NO:23)  
30 P2: 5'-TTTCTCGAGCTGCAATGGTACTTCTGCTC-3'. (SEQ ID NO:24)

The amplified fragment was cloned into pCR-Script Amp SK(+) (Stratagene) according to the manufacturer's protocol. The resulting plasmid was digested with NdeI and Xhol and the fragment corresponding to the cDNA insert from clone 35 wle1n.pk0058.a4 was purified and inserted into the corresponding sites of the *E. coli* expression vector pET24a (Novagen). The resulting plasmid containing the cDNA insert from clone wle1n.pk0058.a4 in the pET24a vector was named pWhtCHL.

Recombinant protein was produced by expression of pWhtCHL in the *E. coli* strain BL21(DE3). A 35 mL culture of cells harboring pWhtCHL was grown in LB media to an absorbance at 600 nm of 0.5 and induction of the recombinant protein was accomplished by addition of IPTG to a final concentration of 0.4 mM. The cells  
5 were grown for an additional 4 hours and harvested by centrifugation. To obtain a crude protein extract, cells were resuspended in 3 mL of lysis buffer (50 mM Tris-HCl (pH7.5), 38  $\mu$ M octylglucoside, and 1 mM PMSF [phenylmethanesulfonyl fluoride]). The cells were then lysed using a French pressure cell. The protein content in the resulting crude extract was determined according to the method of  
10 Bradford (Bradford, M.M. (1976) *Anal. Biochem.* 72: 248-254). Separation of this extract by SDS-polyacrylamide gel electrophoresis and staining with coomasie revealed the presence of a strongly-expressed polypeptide of approximately 35 kDa that was absent from extracts of induced cells harboring the pET24a vector without insert. This 35 kDa induced protein corresponds to the size expected for a  
15 polypeptide derived from the cDNA insert in clone wle1n.pk0058.a4.

The chlorophyllase activity assay was modified from that described by Tsuchiya *et al.* (1997) *Plant Cell Physiol.* 40:104-108). The protein concentration varied depending on whether the assay was done on the vector control or the samples expressing the chlorophyllase. Considerably less protein from the  
20 chlorophyllase samples was used because the activity was much, much greater than that in the vector control. Extracts from *E. coli* expressing the wheat chlorophyllase contained 0.14  $\mu$ g of protein while extracts from *E. coli* containing the vector alone contained 10.5  $\mu$ g of protein. Reactions were conducted in a total volume of 0.5 mL, consisting of 0.4 mL of crude protein prepared above diluted in the lysis buffer and 0.1 mL of a crude chlorophyll extract dissolved in acetone. The concentration of chlorophyll a in the extract was 0.88  $\mu$ M. The assay was  
25 conducted for 5 min with rapid shaking (285 rpm) in order to insure that the acetone phase was dispersed in the reaction buffer. The reaction was stopped with the addition of 1.5 mL of acetone:hexane (1:2, v/v) and 0.5 mL of 2M Tris (pH9.0). After  
30 shaking the reaction tubes to homogenization, the organic and aqueous phases were partitioned by centrifugating the tubes at 2500x g for 10 minutes. The aqueous phase containing the chlorophyllide a reaction product was recovered, and its absorbance was measured at 667 nm. The amount of chlorophyllide a generated in the assay was determined using the extinction coefficient 76.79  $\text{mM}^{-1}\text{cm}^{-1}$ .  
35

From assays conducted as described above, chlorophyllase activity was found to be nearly 900-fold greater in crude extracts of *E. coli* expressing pWhtCHL than in extracts from *E. coli* expressing the pET24a vector without insert. These results are

thus consistent with the functional identification of the cDNA insert in clone wle1n.pk0058.a4 as encoding a chlorophyllase.

**EXAMPLE 8**

**Identification of a Corn Clone Encoding a Different Chlorophyllase Isozyme**

5 The BLASTX search using the EST sequences from clone csc1c.pk006.I9:fis revealed similarity of the polypeptides encoded by the cDNAs to chlorophyllases from *Arabidopsis thaliana* (NCBI General Identifier No. 2460203 and 6729677), *Chenopodium album* (NCBI General Identifier No. 6729675), and *Citrus sinensis* 10 (NCBI General Identifier No. 7328567). Shown in Table 5 are the BLAST results for the sequences of the entire cDNA insert comprising the indicated cDNA clone and encoding entire chlorophyllase ("CGS"):

**TABLE 5**

15 BLAST Results for Sequences Encoding Polypeptides Homologous to Chlorophyllase

Clone	Status	2460203	BLAST pLog Score 6729677	6729675	7328567
csc1c.pk006.I9:fis	CGS	55.15	54.30	47.15	45.52

20 The nucleotide sequence from the entire cDNA insert in clone csc1c.pk006.I9:fis is shown in SEQ ID NO:27 and it encodes an entire corn chlorophyllase. Nucleotides 72 through 1069 from SEQ ID NO:27 encode the amino acid sequence of SEQ ID NO:28 with nucleotides 1070-1072 corresponding to the stop codon. When this corn chlorophyllase is compared with other chlorophyllases it shows an amino acid insertion close to the N-terminus of the protein.

25 Figures 2A-2B present an alignment of the amino acid sequences set forth in SEQ ID NO:28 with the AtCHL1 sequence (SEQ ID NO:19), CaCHL sequence (SEQ ID NO:21), AtCHL2 sequence (SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (SEQ ID NO:22). The lipase and P-loop motifs discussed in Tsuchiya et al. (1999, *Proc. Natl. Acad. Sci. U.S.A.* 96:15362-15367) are 30 underlined and the putative active Serine is written in white and boxed in black. Amino acids identical among all the sequences in the alignment are indicated by an asterisk (\*) above the alignment. As can be seen in the figure, the amino acid sequence of SEQ ID NO:28 contains a motif containing Leu-Ala-Gly-His-Ser-Arg-Gly-Gly-Asp-Thr-Ala-Phe-Ala-Val-Ala-Leu which includes the putative active Serine.

35 The data in Table 6 presents the percent identity of the amino acid sequences set forth in SEQ ID NO:28, with the AtCHL1 sequence (NCBI General Identifier

No. 2460203, SEQ ID NO:21), the CaCHL sequence (NCBI General Identifier No. 6729675, SEQ ID NO:22), the AtCHL2 sequence (NCBI General Identifier No. 6729677, SEQ ID NO:20), and the *Citrus sinensis* chlorophyllase sequence (NCBI General Identifier No. 7328567, SEQ ID NO:22).

5

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Chlorophyllase

Clone	SEQ ID NO.	2460203	6729677	7328567	6729675
csc1c.pk006.19:fis	28	34.0	34.3	28.9	29.1

10

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

15

To determine if the insert in clone csc1c.pk006.19:fis encodes an active chlorophyllase, the encoded polypeptide was expressed in *E. coli* and tested for chlorophyllase activity using essentially the method described in Example 7.

Initially, the chlorophyllase-coding sequence in clone csc1c.pk006.19 was amplified by PCR using *Pfu* polymerase (Stratagene) and the oligonucleotide primers P3 (sense, SEQ ID NO:29) and P4 (antisense, SEQ ID NO:30):

25

P3: 5'-TTCCATGGCGGCATGCCGGTGGC-3' (SEQ ID NO:29)  
 P4: 5'-TTGCGGCCGCCAGCCTTGCTATTCAAGC-3' (SEQ ID NO:30)

The amplified DNA fragment was cloned into pCR-Script Amp SK(+) (Stratagene) according to the manufacturer's protocol. The resulting plasmid was digested with Ncol and NotI and the fragment corresponding to the chlorophyllase-cloning region of clone csc1c.pk006.19:fis was purified and inserted into Ncol/NotI-cut *E. coli* expression vector pET24a (Novagen) to generate the plasmid pZmCHL.

Expression of the protein encoded by clone pZmCHL, its purification, and measurement of chlorophyllase activity will be as described in Example 7.

Sequence alignments, BLAST scores and probabilities, and chlorophyllase activity assays indicate that the nucleic acid fragment comprising the instant cDNA clone encodes one entire corn chlorophyllase isozyme of a class not previously known to Applicant.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising:
  - (a) a nucleotide sequence encoding a polypeptide having chlorophyllase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26 have at least 80% sequence identity, or
  - (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
2. The polynucleotide of Claim 1 wherein the sequence identity is at least 90%.
3. The polynucleotide of Claim 1 wherein the sequence identity is at least 95%.
- 15 4. The polynucleotide of Claim 1 wherein the polynucleotide encodes the polypeptide sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, or 26.
5. The polynucleotide of claim 1 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, or 25.
- 20 6. A vector comprising the polynucleotide of Claim 1.
7. A recombinant DNA construct comprising the polynucleotide of Claim 1 operably linked to at least one regulatory sequence.
8. A cell comprising the recombinant DNA construct of Claim 7.
9. The cell of Claim 8 wherein the cell is a plant cell or a bacterial cell.
10. A method for producing a plant comprising transforming a plant cell with 25 the polynucleotide of Claim 1 and regenerating a plant from the transformed plant cell.
11. A plant comprising the recombinant DNA construct of Claim 7.
12. A seed comprising the recombinant DNA construct of Claim 7.
13. A method for transforming a cell, comprising introducing into a cell the 30 polynucleotide of Claim 1.
14. A method for isolating a polypeptide encoded by the polynucleotide of Claim 1 comprising isolating the polypeptide from a cell transformed with said polynucleotide.

1/8

SEQ ID NO: 19	MAAIEDSPTESS--VVTPAA-----	FEIGSLIPTTEIPVDPV-----	ENDST-----
SEQ ID NO: 20	MS-SSSSR-----	-NAFEDGKYKSNLILTD-----	SSSRASP-----
SEQ ID NO: 22	MAAMVDAKPAAS--VQGTPILLA-----	-TATLPPVFTTRGIYSTKRITESSPS-----	SP-----
SEQ ID NO: 21	MAKLLLLIFGVFIVNSQAQTFTILEKHNSEKITDVFHKGNFQVTNNPI-----	-RVKRYEF-----	
SEQ ID NO: 2	-	-RVET-----	SNIA-----
SEQ ID NO: 4	STCSTTAA-----	-NVFEIGKHITVLLRAE-----	C--TTKSSLPV
SEQ ID NO: 6	MNLASAVRVFLSYLLVQRWMGS-----	EQGGGVFDQGGHSVSLTRLDEARAPPRCAVRSSPSSAAS-----	
SEQ ID NO: 8	MAQRAEPILVT-----	TDVFQMGNIKWKOFNIDT-----	SNASS-----
SEQ ID NO: 10	MAQRAOPVLAT-----	-DVFQMGNIQWKOFNVDT-----	SSASF-----
SEQ ID NO: 12	MAQRAQPALAT-----	TDVFQKQDIHWKQFNVET-----	STASS-----
SEQ ID NO: 14	MASSSPCS-----	--VFVPGKYTVQLKSVE--AGTK--KARHVSSVSA-----	
SEQ ID NO: 16	-		
SEQ ID NO: 18	MAAAAPAETMNK--SAAGAEVP-----	EAFTSVFQPGKLAVEATIQVDE-----	NAAP-----
SEQ ID NO: 26	MAAMA-----	TTVFOAGPMEVDVKHVDK-----	SMIP-----

FIG. 1A

\* \* \* \* \* APPKPVRITCPTVAGTYPVVLFFHGYIIRNYFYSVLNHIASHGYIILVAPOLCKLLPP--GGQ--VEVDDA  
 SEQ ID NO: 19 APPKQOLLVATPVEEGDYPVVMLLHGYLINNSFYSQMLHVSSHGFILIAPOLYSIAGP--DTM--DEIKST  
 SEQ ID NO: 20 SPPKQOLLVATPVEEGDYPVVMLLHGYLINNSFYSQMLHVSSHGFILIAPOLYSIAGP--DTM--DEIKST  
 SEQ ID NO: 22 PPPKPLIIVTPAGKGTENVILFHGTSLNSKSYSKIFDHIASHGFIVVAPOLYSSPP--SAT--NEILNSA  
 SEQ ID NO: 21 SAPEPLIIISPKEAGVYPVLLIFHGTMLSNEYSLFENYIASHHGFIVVAPOLYSSPP--SAT--NEILNSA  
 SEQ ID NO: 21 SPPKPLIIVTPTIQGTYPVLLFLHGFELRNTFTYQLQLISSHHGFIVVAPOLYGLLPP--SGI--QEIKSA  
 SEQ ID NO: 2 SPPKPLIIVTPTIQGTYPVLLFLHGFELRNTFTYQLQLISSHHGFIVVAPOLYGLLPP--SGI--QEIKSA  
 SEQ ID NO: 4 PPLQQLIATPSEAGEFPLLLLHGYLINNSFYSOLIQLQHIAASHGFIVVAPOLYVAGP--DSS--EEIKSA  
 SEQ ID NO: 6 LPPKPLLVAAPRETEGYPVILFHGYLAVNSFYSQLEHVASHGFIVVGPOLYTIAGA--DTT--EEINSA  
 SEQ ID NO: 8 SPPKPLLIIFTPTVPGSYPVILFCGESLIRNSYYSELLGHIAASHGFIIIVAPOLCWSVRSMLLEPG--DEVKFZ  
 SEQ ID NO: 10 SPPKPLLIIFTPTVPGAYPVILFHGFEEIRNFYYSKLLAHIVSHGFIIIVAPOLFESNGLPMYGP--TEVEYA  
 SEQ ID NO: 12 SPPKPLLIIFTPTVPGLYPVILFHGFCEIRTSYYSKLLAHIVSHGFIIIVAPOLFESIGVPMFGP--EEVKCE  
 SEQ ID NO: 14 PPKPPLIATPFEEGEYPTILLHGFMHLNTFYSELQHIAASHGFIVVVPOLYLVATC--DST--NGIKSA  
 SEQ ID NO: 16 -----  
 SEQ ID NO: 18 TPPIPVLIVAPKDAGTYPVAMLLHGFFELHNHFYEHLLRHVASHGFIIIVAPQ--FSISIIPSGDA--EDIAAA  
 SEQ ID NO: 26 NLARPLMVVAPKETGAYPVIVFLHGWNMILNSWYEQLLTHVASHGFIAVAPOLYWMVSEP--DA-DDIDAT

140

71

FIG. 1B

3/8

SEQ	ID	NO:19	GSVINW-ASE--NLIKHLPT-----SYNANGKYTSLVGH	S	RGGKTAFAVALGHAAT-LDPSITESALIGI
SEQ	ID	NO:20	AEIMDWLSV---GLNHFLPA-----QVTPNLSKFALSGH	S	RGGKTAFAVALKKFGY-S-SNLKISTLIGI
SEQ	ID	NO:22	AEVAEWLPO---GLQONLPE-----NTEANVSLVAVMGH	S	RGGQTAFAVALSILRYG-----FGAVIGL
SEQ	ID	NO:21	ASVANWMPLY---LQVVLQRYVT--GVEGDLEKLAISGH	S	RGGKSAFALALGFSNIK--LDVTE SALIGV
SEQ	ID	NO: 2	AAVTNWLISS---GLQSVLPE-----NVKPDLKKLALS GH	S	RGGKTAFAVALALGYADT---SLENFALLGL
SEQ	ID	NO: 4	AALTNWLSK---GLHDLILPP---HVRPNLSKGLAGH	S	RGGKTAFAVALRK--A-S-TSLKFSA LIGI
SEQ	ID	NO: 6	AAVIDDWLAT---GLPSTLPL-----GVRANLTKVSISGH	S	RGGKVAFAVALGH-AK-AKLVPLAAVVA V
SEQ	ID	NO: 8	GKVVWDWLAAE---GLQPLLPE-----NVEAKLDKVLVLS GH	S	KGGKTVFAVALGYAKT---NLKFSA LVGI
SEQ	ID	NO:10	GKVADWI-AE--ELQHLLPE-----NVEANLDKVLVLS GH	S	RGGKTVFAVALGHAKT---NLKFSA LVGI
SEQ	ID	NO:12	GRVVDWL-DN--GLQPLLPE-----SVEAKLEKVLVUGH	S	KGGKTAFAVALGYCRT---KLKFSA LIGI
SEQ	ID	NO:14	AKTTDWLKD---GLQDVLPT-----KVRPDLKKLGLSGH	S	RGGKDAFALAL---GY-AKTTLSFSA LIGI
SEQ	ID	NO:16	-	S	RGGQTAFAVALGLGDAKTKLELKFSALIGV
SEQ	ID	NO:18	AKVADWLP-D--GLPSVLPK-----GVEPELSKALAGH	S	RGGHTAFAVSLAIGHAKT---QLTESALIGL
SEQ	ID	NO:26	KRITNWLAHDHKGLAHVLDVKLEHVEPDLSKALAGH	S	RGGQTAFAVALGLGDAKTKLELKFSALIGV

210

141

FIG. 1C

\*\*\*\*\* DPVAGTNKY--IRTDPHILTYKPESELDI-PVAVVGTGLGP-KWNNVMPPCAPTDLNHEEFYKE--CKA  
 SEQ ID NO:19 DPVAGTGVGK--QTRPPPVAYLPNSFDLDTPLVIGSGLGETARNPLFPPCAPPGVNHREFFRE--CQG  
 SEQ ID NO:20 DPVAGTSKTTG--LDPSILS--DSFDFSI-PVTVIGTGLGGVAR--CITACAPEGANHEEFNRC--CKN  
 SEQ ID NO:22 DPVAGTSSKTTG--LDPSILS--DSFDFSI-PVTVIGTGLGGVAR--CITACAPEGANHEEFNRC--CKN  
 SEQ ID NO:21 DPVAG--RSVDDRTLPHVLTYPNFSNLSI-PVTVIGTGLGNHTIS----CAPNHVSHOOFYDE--CKE  
 SEQ ID NO: 2 DPVGGLSSRC--CQTVPKILTYVPHSFNLAI-PVCVIGTGLGDEPRNCLTCPAPDGVNHVEFFSE--CKP  
 SEQ ID NO: 4 DPVAGMDKGK--QTTPPPVLTYPHSFNLDMA--VMMVIGSGLGEVKRNPPLFPPCAPPKGVNHEDEFKE--CRE  
 SEQ ID NO: 6 DPVAGDMGVGK--QTTPPPVLTGRHGSLLHVG--APAVVIGTGLGELPRGSSLPPCAPRGVSHAAFYDELGAA  
 SEQ ID NO: 8 DPVAGP--CKSCSETFPPLTGMQSFSNLLN--PIVVIIGTGLGPEKANFFIPPCAPDGVNHKEFFNK--CKP  
 SEQ ID NO:10 DPVAGTSKY--CCTRPHILTGKPRSFNLDKM--PVEVIGTGLGPEKLNCCCTPPCAPDGVNYKEFFNE--CKP  
 SEQ ID NO:12 DPVAGVSKCKPCRSLFDILTGVPRSFNLLN--PVAVIIGTGLGPEKANSLEFPPCAPPNGVNHKEFFSE--CKP  
 SEQ ID NO:14 DPVAGVSKCKPCRSLFDILTGVPRSFNLLN--PVAVIIGTGLGELKRN-LFA-CAPKGVNHQDFYDE--CSS  
 SEQ ID NO:16 DPVAGVSRAQ--QLEPKVLTFPDCLDVGM--PVLMGTTGLGPKHIGGF--PCAPVGVNHAEEFYKE--CAP  
 SEQ ID NO:18 DPVAGTGTGK--SSQLQPKILTYEPSSFGMAM--PVLVIGTGLGEEKKNIFFPPCAPKDVNHAEEFYRE--CRP  
 SEQ ID NO:26 DPVAGVSRAQ--QLEPKVLTFPDCLDVGM--PVLMGTTGLGPKHIGGF--PCAPVGVNHAEEFYKE--CAP

211 280

FIG. 1D

\*\*\*\*\*

SEQ ID NO: 19 TK-AHFVAADYGHMDMLDDDLPLGFVG-FMAGCMCKNGQR-KKSEMRSFVGGIVVAVFLKYSLWGEKAEIFL  
 SEQ ID NO: 20 PA-WHFVAKDYGHLDMLDDDTKGIRG-KSSYCYCLCKNGEE--RRPMMRFVGGLVVVSFLKAYLEGDDRELVK  
 SEQ ID NO: 22 SSRAHFVATDYGHMDILDDNPSDVKSUALSKYFCKNGNES-RDPMMRCVSGIVVAVFLKDDFFYGDAEDFRQ  
 SEQ ID NO: 21 NSS-HFVITKYGHMDMLNEFRLSPIAVTMSL-MCAQSFR-PKATMRRTLGGIMVAFLNAYFRDDGRQYYA  
 SEQ ID NO: 2 PC-SHFVTTYEYGHLDMLDDHLSGCGIAI-SGYICKSGK-GPRDPMMRCVGGFLVAVFLKAYLEGQTDGDEKA  
 SEQ ID NO: 4 PA-CYFLAKDYGHLDMLDDETNGIRG-KATHCILCKNGKS--REPMRRFVGGIVIAFMKAYLEGDNSSLIS  
 SEQ ID NO: 6 PA-CHLVVRDYGHDTMMDDDTDPGARG-MLTRTICRSGGA--RAPMRRFVAGATVAVFLKKWVAGDAAMDS  
 SEQ ID NO: 8 PC-AHEFVATEYGHMDMLDDDTPTPLIGISILSNCICKDGK-GPRDLMRRTVGGLVVAVFLRAQLNLWWDENA  
 SEQ ID NO: 10 PC-AKEVVAKYGHMDMLNDDTPLGLICITLVSCKCMCKNGTTGPRDLMRRTGGLVVVSFLRAQLNLWWDENA  
 SEQ ID NO: 12 PS-AYFVATDYGHMDMLDDETPVGIVGTMMSKCMCKNGKKGPRLMRRTVGGLVVAVFLRAQLNEQWKFDA  
 SEQ ID NO: 14 PS-CHEFVAKDYGHMDMLDDDTDGPRG-IVSYCMCRNGES--RTPMRLFVGGIVVAVFLKACLQGDQQLRA  
 SEQ ID NO: 16 PR-YHVVVKDYGHLDMLDDDNVP---YIINNCMCMRNQHDTKDLARRTMGGAMVAFLRAKLRLIDVRLIA  
 SEQ ID NO: 18 PC-YYFVTTKDYGHLDMLDDDAPKFI----TCVCKDGN-GCKGKMRRCVAGIMVAFLNAAALGEKDADLEA  
 SEQ ID NO: 26 PR-YHVVVKDYGHLDMLDDDNVP---YIINNCMCMRNQHDTKDLARRTMGGAMVAFLRAKLRLIDVRLIA

281 350

FIG. 1E

SEQ ID NO: 19 IVKD-PSVSPAKLDPSPLEEASGI----F-----V  
SEQ ID NO: 20 IKDGCHEDVPVEIQEFEV-----M  
SEQ ID NO: 22 ILKD-PSFAPIKILDSVEYIDASSMMLTT-----THVKV  
SEQ ID NO: 21 I-----IANRSLAPTNLFAEKKGGENFGF-----ATTYA-----QL  
SEQ ID NO: 2 IVDE-PDLAPVKLDPVFEFIEA-----V  
SEQ ID NO: 4 IRDG-HATAPVELQNVEFL-----V  
SEQ ID NO: 6 ITAR-PDQAPVALSVVEF-----GDEKAIA  
SEQ ID NO: 8 VLAN-PNLAPTKLDDVVYVPA-----V  
SEQ ID NO: 10 ILKD-PNLAPTEVDGVYDIPA-----M  
SEQ ID NO: 12 ILAS-PNLAPAKLDDVRYLPT-----V  
SEQ ID NO: 14 LKDN-PEISNINLISIVDFYDPDGTLTKYFDGGSISTPHHYAVQESHQ  
SEQ ID NO: 16 IYHN-PEIAPAVLQDVDEFLP-----CFVGRPNPSS-----V  
SEQ ID NO: 18 ILRD-PAVAPTTLDPVEHRVA-----V  
SEQ ID NO: 26 IYHN-PEIAPAVLQDVDEFLP-----CFVGRPNPSS-----V

351 397

FIG. 1F

7/8

MAAI-----EDSPTFSSVVTPAAFEIGSLP-----TTEIPVDPVENDSTAPPKPVIRTCP  
 SEQ ID NO: 19 MSSS-----SSRNAFEDGKYKKSNNLTLSS-----SRCCKITPSSRASPPSPPKQLLIVATP  
 SEQ ID NO: 20 MAAM-----VDAKPAASVQGTPLLATATLPPVFTTRGIYSTKRITLETSSPPSPPKPLIIVTP  
 SEQ ID NO: 21 MAKLLLIFGVFIFVNSQAQTFTILEKHNSEKIT-DVFHKGNFQVTNNPI-RVKRYEFSAPEPLIIISP  
 SEQ ID NO: 28 MAAS-----VAIGTAVFQRGLPLRVEARHVD--YSQVPSVPKPLMVVAP

70

1

-----  
 SEQ ID NO: 19 TVAGTYPVVLFFHGFYLRYNFYSDVLNHIA SHG YILVAPQLCK-LILP--PGGQVEVDDAGSVINWAS---  
 SEQ ID NO: 20 VEEGDYPVVMMLHG YLLNSFYSQMLHVSSHGFILIA PQLYS-IAG--PDTMDEIKSTAEIFDWLS---  
 SEQ ID NO: 22 AGKGTENVILFLHGTSLSNKSYSKIDHIA SHG FIVVAPQLYTSIPP--PSATNELN SAAEVAEWLP---  
 SEQ ID NO: 21 KEAGVYPVLLFIHGTM SNE DYSLFFNYIA SHG FIVVAPKLFLRFLFPKLP SQODEIDMAASVANWMP---  
 SEQ ID NO: 28 TDAGVYPVAVFLHG CNTVNSWYESLLSHVASHGFI A VAPQLYC VTLN--MNDLKDI DATRQVTAWLADKQ

140

71

-----  
 SEQ ID NO: 19 ENLKAHLPT-----SVNAN-GKYTSLVGH S RGGKTAFAVALGHAATL DPS-----ITF  
 SEQ ID NO: 20 VGLNHFLPA-----QVTPNLSKFA-LSGH S RGGKTAFAVAL-KKFGYSSN-----LKI  
 SEQ ID NO: 22 QGLQQQNLP-----NTEANVS-LVAVMGH S RGGQTAFA LSL-----R-----YGF  
 SEQ ID NO: 21 -----LYLQVVLQRYVVTGVEGDL EKL-AISGH S RGGKSAFA ALIGF-----SNIKLDVTF  
 SEQ ID NO: 28 QGLAHVLANILQ--LHGVRPDL SRL-ALAGH S RGGDTAFAVALGLGP AASDDNNADAGTSPAA PLKE

121

180

FIG. 2A

8/8

**	**	**	**	**	**	**	**	**	**
SEQ	ID	No:19	SALIGIDPVAGTNKYIRTDPHILTYKPESEFELD-IPVAVVGTGLGPK-WNNVMPPCAPTDLNHEEFYKEC						
SEQ	ID	No:20	STLIGIDPVDGTKGKQTPPVAYLPLNSFDLDKTPILVIGSGLGETARNPLFPPCAPPGVNHREFFREC						
SEQ	ID	No:22	GAVIGLDPVAGTSKTTGDPISLISF-DSFDFS-IPVTVIGTGLGGVARC--ITACAPEGANHEEFFNRC						
SEQ	ID	No:21	SALIGVDPVAGRSVDDRTLPHVLTYKPNSENLS-IPVTVIGSGLGNHT-----ISCAPNHSVSHQQFYDEC						
SEQ	ID	No:28	SALIGVDPVAGLSKQAQVEPKVLTFRPRSLD-PGMPALVVGTLGP--KHVGGGPPCAPGVNHAEEFYDEC						

SEQ	ID	NO:19	RLIVKD-PSVSPAKLDPSEEAAS-----GIVF
SEQ	ID	NO:20	-VKIKD-GCHE----DVPVEIQE-F-----EVIM
SEQ	ID	NO:22	APIKLDSSVEYIDASSMLTTTHVKVRQILKD-PSF
SEQ	ID	NO:21	YAIIANR-SLAPTNL-FAEKKGFNFATTYQAQL
SEQ	ID	NO:28	KVVLLENRPGLSPAVLDPVG-----HDLA

FIG. 2B

## SEQUENCE LISTING

<110> E.I. du Pont de Nemours and Company

<120> Chlorophyllases

<130> BB1477 PCT

<140>

<141>

<150> 60/238,161

<151> 2000-10-05

<160> 30

<170> Microsoft Office 97

<210> 1

<211> 987

<212> DNA

<213> Vitis sp.

<400> 1

cgagtggaga cttcaaacat cgttccccct cccaaagccat tttgttattgt tacacccaacc 60  
attcaagggc catacccgat tctttgtttt cttcatggct tcggactccg caacaccccttc 120  
tacactcagc tccttcaact catttcttcc catggattca ttgttgggttgc tcctcagttt 180  
tacggactat taccccttcc tggaaattcaa gagatcaaat cagcagcagc agtcacaaat 240  
ttggctatctt caggcccttca atctgtgtcc ccagaaaaatg tggaaaccaga cctactcaag 300  
cttgcgtcttt cagggccacag cagaggggga aagacagcat ttgctctggc actagggat 360  
gctgatacat cccttcaactt ctcagccctt ctggacttag accctgttgg tgggttgagt 420  
aaatgttgc aaacagtttcc caaaatctta acctatgttc ctcattttttt caatctgca 480  
atccccgggtt gcttataatggc caccgggggtt ggcgtatggc caaggaactg cttaaatgt 540  
ccatgtgtccc cagatggagt gaaccatgtt gatgttttca gtgagtgtaa accttcttgc 600  
tccccctttt tgactactga atatggtcac ttggacatgt tagatgtca tcttttcaggc 660  
tgcattgggg cgttttcggg ttatatctgc aagagtggga agggtccttag ggacccctat 720  
aggagatgtt tgggtggcct tttgttgc ttcttggagg cttatgttgg aagttcagact 780  
ggagattca aagccattgt tgatgaacct gatctggctt ctgtgaagct tgatcctgtt 840  
gagttcatag aagcatgaaa tcatggattt tttgttataatgc tataaggagt gtgacagcag 900  
atttttttt cgggtcgctt ttggaaacaa agattcaaat aaacgaaact gtgttgtatt 960  
gctaaaaaaaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 987

<210> 2

<211> 285

<212> PRT

<213> Vitis sp.

<400> 2

Arg Val Glu Thr Ser Asn Ile Ala Ser Pro Pro Lys Pro Leu Leu Ile  
1 5 10 15

Ser Ser His Gly Phe Ile Val Val Ala Pro Gln Leu Tyr Gly Leu Leu  
50 55 60

Pro Pro Ser Gly Ile Gln Glu Ile Lys Ser Ala Ala Ala Val Thr Asn  
 65 70 75 80

Trp Leu Ser Ser Gly Leu Gln Ser Val Leu Pro Glu Asn Val Lys Pro  
 85 90 95

Asp Leu Leu Lys Leu Ala Leu Ser Gly His Ser Arg Gly Gly Lys Thr  
 100 105 110

Ala Phe Ala Leu Ala Leu Gly Tyr Ala Asp Thr Ser Leu Asn Phe Ser  
 115 120 125

Ala Leu Leu Gly Leu Asp Pro Val Gly Gly Leu Ser Lys Cys Cys Gln  
 130 135 140

Thr Val Pro Lys Ile Leu Thr Tyr Val Pro His Ser Phe Asn Leu Ala  
 145 150 155 160

Ile Pro Val Cys Val Ile Gly Thr Gly Leu Gly Asp Glu Pro Arg Asn  
 165 170 175

Cys Leu Thr Cys Pro Cys Ala Pro Asp Gly Val Asn His Val Glu Phe  
 180 185 190

Phe Ser Glu Cys Lys Pro Pro Cys Ser His Phe Val Thr Thr Glu Tyr  
 195 200 205

Gly His Leu Asp Met Leu Asp Asp His Leu Ser Gly Cys Ile Gly Ala  
 210 215 220

Ile Ser Gly Tyr Ile Cys Lys Ser Gly Lys Gly Pro Arg Asp Pro Met  
 225 230 235 240

Arg Arg Cys Val Gly Gly Leu Phe Val Ala Phe Leu Lys Ala Tyr Leu  
 245 250 255

Glu Gly Gln Thr Gly Asp Phe Lys Ala Ile Val Asp Glu Pro Asp Leu  
 260 265 270

Ala Pro Val Lys Leu Asp Pro Val Glu Phe Ile Glu Ala  
 275 280 285

<210> 3  
 <211> 1216  
 <212> DNA  
 <213> Vitis sp.

<400> 3

ttccacatg ttccacact gctgcaaatg ttttgagat tggaaagcac atcacagtgc 60  
 ttctaagagc tgaaccaggc acttgcacca ccaagtcatc tcttcctgtt cccctccac 120  
 tgcaactctt gattgctacg ccctctgaag caggggagtt cccgctgctg ctcttccttc 180  
 atggttatct tctctataac tctttctact cccagctcat ccaacacata gcctctcatg 240  
 gtttcatgt tcttgctcct cagttataca ctgtggctgg accagattca agcgaagaga 300  
 tcaagtccgc agctgcttta acaaatttgt tatccaaagg actccatgac ttacttcctc 360  
 cccatgttcg gccaaattta agcaaactag gacttgcgg ccatagtcgt ggaggcaaaa 420  
 ctgccttgc tctagcactg agaaaagcat ccacttctct gaaatttca gccttgatag 480  
 gcatagaccc ggtggatgga atggacaaag gggaaacaaac ccctccaccc gtactcacct 540  
 atgttcctca ttcatttgat cttagacatgg cagtgtatgg aattggttcg ggtttgggtg 600  
 aagtggaaag gaaccctctg ttccctcctt gtgcccccaa gggcgtaaac catgaggact 660  
 tctttaaaga atgccgtgaa ccagcttgc attttcttgc caaggactat ggcacatctg 720

acatgctaga cgatgagact aatggattt aaggaaaagc tacacattgt ttgtgtaaaa 780  
 atggaaagtc tagagaaccc atgaggaggt ttgttgagg cattgtgatt gcatttatga 840  
 aagcttattt ggaaggcgat aacagcagtc taatctccat tagagatggg catgctactg 900  
 caccagtggaa gcttcaaat gttgagttc tcgtgtgaaa ttttaatgt ttgcatttca 960  
 atagggtggca ttttctagt atgttatgaa tgcccaaagt tttccctta attccaaggc 1020  
 aagatccaac tttattcggta tgcctgccta acaggcaata tcttaatgcc aacattagcc 1080  
 acaacttcta tcaaattcatt ggggttcc cctgatgcc gggcaaatag actctgtttg 1140  
 gatgcttgg tgaactggaaa tatgataatg ccgtgtttgg tgaaaatttt tattagaaaa 1200  
 aaaaaaaaaa aaaaaa 1216

<210> 4  
 <211> 311  
 <212> PRT  
 <213> Vitis sp.

<400> 4  
 Ser Thr Cys Ser Thr Thr Ala Ala Asn Val Phe Glu Ile Gly Lys His  
 1 5 10 15

Ile Thr Val Leu Leu Arg Ala Glu Pro Gly Thr Cys Thr Thr Lys Ser  
 20 25 30

Ser Leu Pro Val Pro Pro Leu Gln Leu Leu Ile Ala Thr Pro Ser  
 35 40 45

Glu Ala Gly Glu Phe Pro Leu Leu Leu Leu His Gly Tyr Leu Leu  
 50 55 60

Tyr Asn Ser Phe Tyr Ser Gln Leu Ile Gln His Ile Ala Ser His Gly  
 65 70 75 80

Phe Ile Val Leu Ala Pro Gln Leu Tyr Thr Val Ala Gly Pro Asp Ser  
 85 90 95

Ser Glu Glu Ile Lys Ser Ala Ala Leu Thr Asn Trp Leu Ser Lys  
 100 105 110

Gly Leu His Asp Leu Leu Pro Pro His Val Arg Pro Asn Leu Ser Lys  
 115 120 125

Leu Gly Leu Ala Gly His Ser Arg Gly Gly Lys Thr Ala Phe Ala Leu  
 130 135 140

Ala Leu Arg Lys Ala Ser Thr Ser Leu Lys Phe Ser Ala Leu Ile Gly  
 145 150 155 160

Ile Asp Pro Val Asp Gly Met Asp Lys Gly Lys Gln Thr Pro Pro Pro  
 165 170 175

Val Leu Thr Tyr Val Pro His Ser Phe Asp Leu Asp Met Ala Val Met  
 180 185 190

Val Ile Gly Ser Gly Leu Gly Glu Val Lys Arg Asn Pro Leu Phe Pro  
 195 200 205

Pro Cys Ala Pro Lys Gly Val Asn His Glu Asp Phe Phe Lys Glu Cys  
 210 215 220

Arg Glu Pro Ala Cys Tyr Phe Leu Ala Lys Asp Tyr Gly His Leu Asp  
 225 230 235 240

Met Leu Asp Asp Glu Thr Asn Gly Ile Arg Gly Lys Ala Thr His Cys  
245 250 255

Leu Cys Lys Asn Gly Lys Ser Arg Glu Pro Met Arg Arg Phe Val Gly  
260 265 270

Gly Ile Val Ile Ala Phe Met Lys Ala Tyr Leu Glu Gly Asp Asn Ser  
275 280 285

Ser Leu Ile Ser Ile Arg Asp Gly His Ala Thr Ala Pro Val Glu Leu  
290 295 300

Gln Asn Val Glu Phe Leu Val  
305 310

<210> 5

<211> 1302

<212> DNA

<213> Zea mays

<400> 5

tcggccacga ttcccagtca tcaccaatta ttactgcctt tcgtccacaa ctactcatca 60  
atccgatcga catgaacacctc gcgtccgcgg tgcgagtgtt ctgtcctac tacctgctgg 120  
tccaacggtg gatggggtcc gagcagggcg gaggcgtgtt cgatcagggg ggccatagcg 180  
tcagcctcac cccgcctcgac gaagcaaggg cgccgcgcgg gtgcgcgtg cggcgtccc 240  
cgtcagcgcg ggcgcgcctg cgcgcgaagc cgctgctcgt cgccgcgcgg cgcgagactg 300  
gggagtagccc ggtgatcctg ttccctacacg gctacctcgc cgtcaactcc ttctactccc 360  
agctgttgcg gacacgtcgcc tcccattggct ttatcgtcgt cggacctcag ctgtacacca 420  
tatctggggc cgacaccacc gaggagatca actcagcggc ggccgtcata gactggctag 480  
ccacccggct ggcgtcaact ctggcactcg gcgtccgcgc gaaacctaacc aagggtgtcca 540  
tctccggcca cagtcgcggc gggaaagggtgg cgttcgcgcgt ggcgttggc caccgcaagg 600  
ccaaagctcgc tgcccccttc gccgcgcgtcg tgcgcgtggc cccgggtggac ggcattggcg 660  
tggccaagca gacaccgcgc cgcatttcgc cgggcaggca cggcgcgtcg cacgtgggtg 720  
ccccccgcatt ggtcatcgcc acggggctcg gcgcgcgtcgc cccggcgtca ctgcgtccgc 780  
cggtgcgcgc cccggggcgtc acgtcacgcgg ccttctacga cgagctggac ggcgcggcgc 840  
cagcgtgcca cctgggtggc agggactacg ggacacacggc catgtggac gacgacacgc 900  
cggcgcgcag ggggatgctc acgcgcacca tctgcaggag cggcggggcc agggcgccca 960  
tgcggcgtt cgtggccggc gcccaccgtcg cgttcctcaa gaaatgggtg gctggggacg 1020  
ccggccgcgt gacagcata acggcgcggc cgacccaggc cccgcgcgc ctgtccgtgg 1080  
tggagtttgg agatggaaaa ggcatacgatcgtt agtgcgttga gaatatgtga tatctgcagg 1140  
caactggccac tcaggcaggat tggtgcacgc gttgaaattt cagatggaaat taacttagat 1200  
ctagagatgg cacaatggcag attggcagtg agtttcgaaa ctagctaatt tcctctctct 1260  
tggcaaatca aataaaaaaaa aaaaaaaaaaa aaaaaaaaaaa aa 1302

<210> 6

<211> 346

<212> PRT

<213> Zea mays

<400> 6

Met Asn Leu Ala Ser Ala Val Arg Val Phe Leu Ser Tyr Tyr Leu Leu  
1 5 10 15

Val Gln Arg Trp Met Gly Ser Glu Gln Gly Gly Gly Val Phe Asp Gln  
20 25 30

Gly Gly His Ser Val Ser Leu Thr Arg Leu Asp Glu Ala Arg Ala Pro  
35 40 45

Pro Arg Cys Ala Val Arg Ser Ser Pro Ser Ser Ala Ala Ser Leu Pro  
 50 55 60

Pro Lys Pro Leu Leu Val Ala Ala Pro Arg Glu Thr Gly Glu Tyr Pro  
 65 70 75 80

Val Ile Leu Phe Leu His Gly Tyr Leu Ala Val Asn Ser Phe Tyr Ser  
 85 90 95

Gln Leu Phe Glu His Val Ala Ser His Gly Phe Ile Val Val Gly Pro  
 100 105 110

Gln Leu Tyr Thr Ile Ser Gly Ala Asp Thr Thr Glu Glu Ile Asn Ser  
 115 120 125

Ala Ala Ala Val Ile Asp Trp Leu Ala Thr Gly Leu Pro Ser Thr Leu  
 130 135 140

Pro Leu Gly Val Arg Ala Asn Leu Thr Lys Val Ser Ile Ser Gly His  
 145 150 155 160

Ser Arg Gly Gly Lys Val Ala Phe Ala Leu Ala Leu Gly His Ala Lys  
 165 170 175

Ala Lys Leu Ala Val Pro Leu Ala Ala Val Val Ala Val Asp Pro Val  
 180 185 190

Asp Gly Met Gly Val Gly Lys Gln Thr Pro Pro Pro Ile Leu Thr Gly  
 195 200 205

Arg His Gly Ser Leu His Val Gly Ala Pro Ala Met Val Ile Gly Thr  
 210 215 220

Gly Leu Gly Glu Leu Pro Arg Gly Ser Leu Leu Pro Pro Cys Ala Pro  
 225 230 235 240

Arg Gly Val Ser His Ala Ala Phe Tyr Asp Glu Leu Asp Gly Ala Ala  
 245 250 255

Pro Ala Cys His Leu Val Val Arg Asp Tyr Gly His Thr Asp Met Met  
 260 265 270

Asp Asp Asp Thr Pro Gly Ala Arg Gly Met Leu Thr Arg Thr Ile Cys  
 275 280 285

Arg Ser Gly Gly Ala Arg Ala Pro Met Arg Arg Phe Val Ala Gly Ala  
 290 295 300

Thr Val Ala Phe Leu Lys Lys Trp Val Ala Gly Asp Ala Ala Ala Met  
 305 310 315 320

Asp Ser Ile Thr Ala Arg Pro Asp Gln Ala Pro Val Ala Leu Ser Val  
 325 330 335

Val Glu Phe Gly Asp Glu Lys Ala Ile Ala  
 340 345

<210> 7  
 <211> 1174

&lt;212&gt; DNA

&lt;213&gt; Glycine max

&lt;400&gt; 7

ggaaagtgtt gatggcgac agagctgaac caaatattgtt caccacagat gttttccaaa 60  
 tggaaatataat caaatggaaag caattcaaca ttgatacata caatgttcc tcctcac  
 caaaaaccatt gttaatctt acaccaaccg tgcctggctc ataccctgtt atattgttct 120  
 gccatggatt ttcccttcgc aatagctact actctgagct cctaggccac atagcttac 180  
 atggattcat aattgttgc cctcagctgt gttggagtgt acggctatg ttgaaacctg 240  
 gtgtgaagt taaaatttgcg gggaaagtgtt tgattttggctt agccgaggag gggcttcaac 300  
 ctctgttcc agagaatgtt gaagccaaat tggataaattt ggttttatca ggtcacagca 360  
 agggggcaaa aactgtattt gctgtggcac ttggttatgc taaaactaac ctcaagttt 420  
 cagcactagt aggcatacagc cctgtggctg gcccatgtaa atcttgcgaa acatttcctc 480  
 ctatctcac tggcatgttcc caatcttca atttgaacat acccattgtt gtaattggca 540  
 ctgggcttgggcccagagaag gctaaatttt ttattccacc atgtgttcc gatgggggtga 600  
 accataagga gtttttcaat aagtgcacccccccttgc acatttttt gcaactgagt 660  
 atggtcacat ggacatgtt gatgtgtga cacctggctt aattgggtca atattgtcaa 720  
 attgtatatg caaggatggg aagggtccta gggacttgat gagaaggacc gtgggggggt 780  
 tgggtgtggc cttcttaagg gcacagttga atggcctatg gaaggatttt aatgctgttt 840  
 tggcgaatcc taatcttgc tctactaaac tggatgtgt agtgtacgta cccgcatgaa 900  
 gtccataactc aatattaaag gctcttgggt tcgatgtgttca attgcttca atgccaagg 960  
 ttcttttcaaa agaaatcaag tttcttcgat gatgtttgtt tttgtttcc ataaaaggct 1020  
 tgtaatggaa attttaaaat aatcttctat tggttacatt ctctgtcgag ttttacgtg 1080  
 ggataatgtt tattcaaaaa aaaaaaaaaaaa aaaa 1140  
 1174

&lt;210&gt; 8

&lt;211&gt; 315

&lt;212&gt; PRT

&lt;213&gt; Glycine max

&lt;400&gt; 8

Met Ala Gln Arg Ala Glu Pro Ile Leu Val Thr Thr Asp Val Phe Gln  
 1 5 10 15

Met Gly Asn Ile Lys Trp Lys Gln Phe Asn Ile Asp Thr Ser Asn Ala  
 20 25 30

Ser Ser Ser Pro Pro Lys Pro Leu Leu Ile Phe Thr Pro Thr Val Pro  
 35 40 45

Gly Ser Tyr Pro Val Ile Leu Phe Cys His Gly Phe Ser Leu Arg Asn  
 50 55 60

Ser Tyr Tyr Ser Glu Leu Leu Gly His Ile Ala Ser His Gly Phe Ile  
 65 70 75 80

Ile Val Ala Pro Gln Leu Cys Trp Ser Val Arg Ser Met Leu Glu Pro  
 85 90 95

Gly Asp Glu Val Lys Phe Ala Gly Lys Val Val Asp Trp Leu Ala Glu  
 100 105 110

Glu Gly Leu Gln Pro Leu Leu Pro Glu Asn Val Glu Ala Lys Leu Asp  
 115 120 125

Lys Leu Val Leu Ser Gly His Ser Lys Gly Gly Lys Thr Val Phe Ala  
 130 135 140

Val Ala Leu Gly Tyr Ala Lys Thr Asn Leu Lys Phe Ser Ala Leu Val  
 145 150 155 160

Gly Ile Asp Pro Val Ala Gly Pro Cys Lys Ser Cys Glu Thr Phe Pro  
 165 170 175

Pro Ile Leu Thr Gly Met Ser Gln Ser Phe Asn Leu Asn Ile Pro Ile  
 180 185 190

Val Val Ile Gly Thr Gly Leu Gly Pro Glu Lys Ala Asn Phe Phe Ile  
 195 200 205

Pro Pro Cys Ala Pro Asp Gly Val Asn His Lys Glu Phe Phe Asn Lys  
 210 215 220

Cys Lys Pro Pro Cys Ala His Phe Val Ala Thr Glu Tyr Gly His Met  
 225 230 235 240

Asp Met Leu Asp Asp Val Thr Pro Gly Leu Ile Gly Ser Ile Leu Ser  
 245 250 255

Asn Cys Ile Cys Lys Asp Gly Lys Gly Pro Arg Asp Leu Met Arg Arg  
 260 265 270

Thr Val Gly Gly Leu Val Val Ala Phe Leu Arg Ala Gln Leu Asn Gly  
 275 280 285

Leu Trp Lys Asp Phe Asn Ala Val Leu Ala Asn Pro Asn Leu Ala Pro  
 290 295 300

Thr Lys Leu Asp Asp Val Val Tyr Val Pro Ala  
 305 310 315

<210> 9

<211> 1104

<212> DNA

<213> Glycine max

<400> 9

gcacgagtgt gagaagagtg gtgtgatggc gcagagagct caaccaggat tggccacaga 60  
 tggccacaa atggaaaca tccaaatggaa gcaattcaat gttgatacat ccagtgccttc 120  
 ctttcaccc tccaaaccat tgctaatttt tacaccaact gttcctggcg cataccctgt 180  
 aatattgttc gtccatggct tttcattcg caatttctac tactcaaagc tcctagccca 240  
 catagtctca catggattca taatcggtc tcctcaactg ttttccaatg ggcttccttat 300  
 gtatggaccc actgaagtgg aatatgcagg aaaagttgcg gattggatag ctgaggagct 360  
 tcaacatttg cttccagaga acgttgaaagc caatttggac aaactgggtc tatcaggtca 420  
 cagtaggggt gggaaaactg tatttgcgtg ggcttgggt catgcaaaaa ctaatctcaa 480  
 gtttcagca cttgttaggca tagaccctgt ggctggcaca tctaaatatt gtagaacacg 540  
 tcctcatatt ctcactggca agccacggtc cttgatttg aaaatgccag ttgaagtaat 600  
 tggcacttggaa ttggggccca agaagttaa ttgttgcgtact ccaccgtgtg ctcctgtatgg 660  
 ggtgaactat aaggagttct tcaacgagtg caaacccttct tgcgttaat ttgttgcgt 720  
 aaagttatggt cacatggaca tggtaatga tgacacacca gggctaattg ggacattgg 780  
 gtcaaaatgtt atgtgttaaga atgggacgac ggttgcgttgg gacttgcgtgaaaggaccac 840  
 tggagggttg ttgtgtccct tcttggggc acaatttgaat gacccatggaaaggatgg 900  
 tgctatatttta aaggacccta atcttgcgtcc cactgaaatgc gatgggtgttag actacataacc 960  
 agcatgaattt gatcaacactt caacgttctt ttgcgtttt atttcccttga aaggccctata 1020  
 aatgtcttca gaaataatcg tctatggta ttatgtattt ctcgttatcc tgcgtatgtat 1080  
 taagaaaaaaa aaaaaaaaaaaa aaaa 1104

<210> 10

<211> 313

&lt;212&gt; PRT

&lt;213&gt; Glycine max

&lt;400&gt; 10

Met	Ala	Gln	Arg	Ala	Gln	Pro	Val	Leu	Ala	Thr	Asp	Val	Phe	Gln	Met
1															
															15

Gly	Asn	Ile	Gln	Trp	Lys	Gln	Phe	Asn	Val	Asp	Thr	Ser	Ser	Ala	Ser
															30
20								25							

Phe	Ser	Pro	Pro	Lys	Pro	Leu	Leu	Ile	Phe	Thr	Pro	Thr	Val	Pro	Gly
															45
35								40							

Ala	Tyr	Pro	Val	Ile	Leu	Phe	Val	His	Gly	Phe	Phe	Ile	Arg	Asn	Phe
															50
															55

Tyr	Tyr	Ser	Lys	Leu	Leu	Ala	His	Ile	Val	Ser	His	Gly	Phe	Ile	Ile
															65
															70

Val	Ala	Pro	Gln	Leu	Phe	Ser	Asn	Gly	Leu	Pro	Met	Tyr	Gly	Pro	Thr
															85
															90

Glu	Val	Glu	Tyr	Ala	Gly	Lys	Val	Ala	Asp	Trp	Ile	Ala	Glu	Glu	Leu
															100
															105

Gln	His	Leu	Leu	Pro	Glu	Asn	Val	Glu	Ala	Asn	Leu	Asp	Lys	Leu	Val
															115
															120

Leu	Ser	Gly	His	Ser	Arg	Gly	Gly	Lys	Thr	Val	Phe	Ala	Val	Ala	Leu
															130
															135

Gly	His	Ala	Lys	Thr	Asn	Leu	Lys	Phe	Ser	Ala	Leu	Val	Gly	Ile	Asp
															145
															150

Pro	Val	Ala	Gly	Thr	Ser	Lys	Tyr	Cys	Arg	Thr	Arg	Pro	His	Ile	Leu
															165
															170

Thr	Gly	Lys	Pro	Arg	Ser	Phe	Asp	Leu	Lys	Met	Pro	Val	Glu	Val	Ile
															180
															185

Gly	Thr	Gly	Leu	Gly	Pro	Glu	Lys	Leu	Asn	Cys	Cys	Thr	Pro	Pro	Cys
															195
															200

Ala	Pro	Asp	Gly	Val	Asn	Tyr	Lys	Glu	Phe	Phe	Asn	Glu	Cys	Lys	Pro
															210
															215

Pro	Cys	Ala	Lys	Phe	Val	Val	Ala	Lys	Tyr	Gly	His	Met	Asp	Met	Leu
															225
															230

Asn	Asp	Asp	Thr	Pro	Gly	Leu	Ile	Gly	Thr	Leu	Val	Ser	Lys	Cys	Met
															245
															250

Cys	Lys	Asn	Gly	Thr	Thr	Gly	Pro	Arg	Asp	Leu	Met	Arg	Arg	Thr	Thr
															260
															265

Gly	Gly	Leu	Val	Val	Ser	Phe	Leu	Arg	Ala	Gln	Leu	Asn	Asp	Leu	Trp
															275
															280

Lys	Asp	Phe	Asp	Ala	Ile	Leu	Lys	Asp	Pro	Asn	Leu	Ala	Pro	Thr	Glu
															290
															295

Val Asp Gly Val Asp Tyr Ile Pro Ala  
305 310

<210> 11  
<211> 1125  
<212> DNA  
<213> Glycine max

<400> 11  
tagaacatag aagcaaagtg tttgggtgtga gaagagagat aatggcgcaag agagctcaac 60  
cagcggtggc caccacggat gttttcaga agggagatat tcatttgaag caattcaatg 120  
ttgaaacatc cactgcttct tcctcacatc caaaaccatt gcttattttt acaccaaccg 180  
tgcctggctt ataccctgtt atattttttt gccatggctt ttgtatttgc actagctact 240  
actctaagct cctagccac atagttcac atggattcat acttggcttct cctcagctgt 300  
tttccattgg ggtgcctatg tttggaccag aagaagttaa gtgtgaagga agagttgtgg 360  
attggctaga taacgggctt caaccattgc ttcccgagag cggtgaagcc aaactggaga 420  
aactggttct agtaggtcac agcaagggtg gaaaaacagc atttgcgtg gcaacttggtt 480  
actgtaaaac aaagctcaag ttttcagcac tcataggcat agatccctgt gctggcgat 540  
caaagtgtaa gccttgcga tcacttcttgc atatcctcac aggtgtgcac cggcccttta 600  
atctgaacat acctgttgcgt gtaattggaa ctgggttggg cccagagaag gctaatttctc 660  
ttttccacc atgtgcctca aatgggtga accataaaa gtttttcttgcgt gatgtcaaac 720  
cacctagtgc atattttgtt gcaacggatt atggtcacat ggacatgttgcgt gatgtgaaa 780  
caccagggtt aattgggaca atgatgtcaa agtgtatgtg caagaatggg aagaagggtc 840  
ctaggactt gatgagaagg actgtgggag ggttgggtgt ggccttcttgcgt gacacagt 900  
tgaatgagca gtggaaaggat tttgatgtca ttttagcgag tcctaattcttgcgt 960  
aacttgatga tttgcatac ttaccaacat gaagtcatca cacaacatt catttatgca 1020  
gttttatttc tatttctatg aaaggcctat aatgtcttca aatgttgcgt atataatgat 1080  
gtgattcaca ttgcactttt caaaaaaaaaaaaaaaac tcgag 1125

<210> 12  
<211> 316  
<212> PRT  
<213> Glycine max

<400> 12  
Met Ala Gln Arg Ala Gln Pro Ala Leu Ala Thr Thr Asp Val Phe Gln  
1 5 10 15

Lys Gly Asp Ile His Trp Lys Gln Phe Asn Val Glu Thr Ser Thr Ala  
20 25 30

Ser Ser Ser Pro Pro Lys Pro Leu Leu Ile Phe Thr Pro Thr Val Pro  
35 40 45

Gly Leu Tyr Pro Val Ile Leu Phe Cys His Gly Phe Cys Ile Arg Thr  
50 55 60

Ser Tyr Tyr Ser Lys Leu Leu Ala His Ile Val Ser His Gly Phe Ile  
65 70 75 80

Leu Val Ala Pro Gln Leu Phe Ser Ile Gly Val Pro Met Phe Gly Pro  
85 90 95

Glu Glu Val Lys Cys Glu Gly Arg Val Val Asp Trp Leu Asp Asn Gly  
100 105 110

Leu Gln Pro Leu Leu Pro Glu Ser Val Glu Ala Lys Leu Glu Lys Leu  
115 120

Val Leu Val Gly His Ser Lys Gly Gly Lys Thr Ala Phe Ala Val Ala  
 130 135 140

Leu Gly Tyr Cys Lys Thr Lys Leu Lys Phe Ser Ala Leu Ile Gly Ile  
 145 150 155 160

Asp Pro Val Ala Gly Val Ser Lys Cys Lys Pro Cys Arg Ser Leu Pro  
 165 170 175

Asp Ile Leu Thr Gly Val Pro Arg Ser Phe Asn Leu Asn Ile Pro Val  
 180 185 190

Ala Val Ile Gly Thr Gly Leu Gly Pro Glu Lys Ala Asn Ser Leu Phe  
 195 200 205

Pro Pro Cys Ala Pro Asn Gly Val Asn His Lys Glu Phe Phe Ser Glu  
 210 215 220

Cys Lys Pro Pro Ser Ala Tyr Phe Val Ala Thr Asp Tyr Gly His Met  
 225 230 235 240

Asp Met Leu Asp Asp Glu Thr Pro Gly Val Ile Gly Thr Met Met Ser  
 245 250 255

Lys Cys Met Cys Lys Asn Gly Lys Lys Gly Pro Arg Asp Leu Met Arg  
 260 265 270

Arg Thr Val Gly Gly Leu Val Val Ala Phe Leu Arg Ala Gln Leu Asn  
 275 280 285

Glu Gln Trp Lys Asp Phe Asp Ala Ile Leu Ala Ser Pro Asn Leu Ala  
 290 295 300

Pro Ala Lys Leu Asp Asp Val Arg Tyr Leu Pro Thr  
 305 310 315

<210> 13  
 <211> 1444  
 <212> DNA  
 <213> Gesneriana

<400> 13

gcacgaggca gaatagacat tgttttttt cttttttgaa aattgccaac cctggggaggt 60  
 gttgacgggg ttaagtccag actagacatt gttatcatgg catcttc tttgtccgtt 120  
 tttgtccctg ggaagtacac ggtccaaactg aaaagcgtcg aggccgcac taagaaggcc 180  
 aggcatgtta gctctgtctc cgcaccaccc cggaaagccac cactgatcgc aactccattt 240  
 gaggaaggcg agtacccaaac gcttctactc cttcatggat tcatgcttca caacactttc 300  
 tactctgagc ttatccagca catcgatcc catggctta ttgttgttgc acctcagttt 360  
 tatctttagt ctacatgtga tagtacgaat ggcatacgat ctgctgaaa aacaacagat 420  
 tgggttgaagg atggactgca agatgttctc ccaacaaaaag tcagaccaga cctaaagaaa 480  
 ctggactgaa gccggacatag ccgtggccgc aaagatgcat ttgtcttgc actaggatat 540  
 gcgaaagacta cattaagctt ctcagcgctg atcgggatcg atcctgttga cggagttacga 600  
 aaggagacacc aaaccaatcc tcctgtactg aatttatatcc ctcactctt ggaactcaag 660  
 atggccgtcat tagttatccg aacaggttta ggtgaattga agaggaacctt atttgcctgt 720  
 gcccccaagg gagtaaatca ccaagattt tacatgagt ttgttccccc gtcttgccat 780  
 ttgttgcata aggactatgg tcacatggac atgcttagatg atcatactga cggacccatg 840  
 ggtattgtgt catactgcat gtgtcggaaat ggagaatcaa gaacacccat gcgactgtt 900  
 gtaggtggaa tcgtgggtgc ttttctgaaa gcctgcttac aaggtgataa acaggatttg 960  
 cgagctctga aggataaccc cgagatttca aatattactt tatcaattgt cgacttcttat 1020

gatcctgatg ggacattgac gaaatatttt gatggtggtt caatttccac accacattac 1080  
 gcagtccaaag aaagccatca acaatgatat ttcaatgcag agacgcacaa ctaatgattg 1140  
 cctgcaaata tgaagttccg tattagattc atataatgaa ctaggatatac tctttcatct 1200  
 aaatagtcca tgtatttat ttcatgcaga gtatgtttaa tctatgtaat aagagacact 1260  
 actagtatct atcgtcagca gcatggcaat ttaaaactct gtaaaactctc agaattatga 1320  
 ttgtatcttgc gcggttagcc ttgacaaaaaa agagaggcac tattagcaag tggaaaaaaaa 1380  
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1440  
 aaaa 1444

<210> 14  
 <211> 336  
 <212> PRT  
 <213> Gesneriana

<400> 14  
 Met Ala Ser Ser Pro Cys Ser Val Phe Val Pro Gly Lys Tyr Thr Val  
 1 5 10 15  
 Gln Leu Lys Ser Val Glu Ala Gly Thr Lys Lys Ala Arg His Val Ser  
 20 25 30  
 Ser Val Ser Ala Pro Pro Arg Lys Pro Pro Leu Ile Ala Thr Pro Phe  
 35 40 45  
 Glu Glu Gly Glu Tyr Pro Thr Leu Leu Leu His Gly Phe Met Leu  
 50 55 60  
 His Asn Thr Phe Tyr Ser Glu Leu Ile Gln His Ile Ala Ser His Gly  
 65 70 75 80  
 Phe Ile Val Val Val Pro Gln Leu Tyr Leu Val Ala Thr Cys Asp Ser  
 85 90 95  
 Thr Asn Gly Ile Lys Ser Ala Ala Lys Thr Thr Asp Trp Leu Lys Asp  
 100 105 110  
 Gly Leu Gln Asp Val Leu Pro Thr Lys Val Arg Pro Asp Leu Lys Lys  
 115 120 125  
 Leu Gly Leu Ser Gly His Ser Arg Gly Gly Lys Asp Ala Phe Ala Leu  
 130 135 140  
 Ala Leu Gly Tyr Ala Lys Thr Thr Leu Ser Phe Ser Ala Leu Ile Gly  
 145 150 155 160  
 Ile Asp Pro Val Asp Gly Val Arg Lys Gly His Gln Thr Asn Pro Pro  
 165 170 175  
 Val Leu Asn Tyr Ile Pro His Ser Leu Glu Leu Lys Met Pro Ser Leu  
 180 185 190  
 Val Ile Gly Thr Gly Leu Gly Glu Leu Lys Arg Asn Leu Phe Ala Cys  
 195 200 205  
 Ala Pro Lys Gly Val Asn His Gln Asp Phe Tyr Asp Glu Cys Ser Ser  
 210 215 220  
 Pro Ser Cys His Phe Val Ala Lys Asp Tyr Gly His Met Asp Met Leu  
 225 230 235 240

Asp Asp His Thr Asp Gly Pro Arg Gly Ile Val Ser Tyr Cys Met Cys  
 245 250 255

Arg Asn Gly Glu Ser Arg Thr Pro Met Arg Leu Phe Val Gly Gly Ile  
 260 265 270

Val Val Ala Phe Leu Lys Ala Cys Leu Gln Gly Asp Lys Gln Asp Leu  
 275 280 285

Arg Ala Leu Lys Asp Asn Pro Glu Ile Ser Asn Ile Asn Leu Ser Ile  
 290 295 300

Val Asp Phe Tyr Asp Pro Asp Gly Thr Leu Thr Lys Tyr Phe Asp Gly  
 305 310 315 320

Gly Ser Ile Ser Thr Pro His Tyr Ala Val Gln Glu Ser His Gln Gln  
 325 330 335

<210> 15

<211> 916

<212> DNA

<213> Triticum aestivum

<400> 15

ccacgagtcg agcctgacct gtccaaagctg gccctagccg gccatagccg agggggccag 60  
 acggccttcg ccgtcgccct gggactaggg gacgccaaga ccaagcttga gctcaagttc 120  
 tccgcctca tcggcgtcga ccccggtggcc ggggtttcca gagcccaaca gttggagccc 180  
 aaggtgctca ctttgaacc tgactgtc tgcgtgggg tgccgggtct ggtcatgggg 240  
 actgggctgg gtcccaagca catcgccgga ttccatgcg ccccggtggg cgtgaaccac 300  
 gccaattct acaaggagtg cgccgcgcct cgctaccacc tcgtggtcaa ggactacggg 360  
 catctcgaca tgctggatga caatgtgcc tatatcatca acaactgcat gtgcattgagg 420  
 aaccaacacg acaccaaaga tcttgcatttggg aggaccatgg gaggagccat ggttgccttc 480  
 ctcagggtca aattgcgaat cgatgttcgt gatctcatcg ccatatatca taatcctgag 540  
 atcgcgcgc cctgtcctggc ccaagttgtatggg gagtttcttc cttgcttcgt tggacggcca 600  
 aatccgtcgt ctgtgtgaga gtttatatttc gtgcctatgc cttatcttata ctcctaaata 660  
 aggtgtgtt gtgcattggcc ttgtcatggc tcattactac tatagcacgt ccagtcttcc 720  
 taattactaa ataataagca ctaatgtcat gtgtgcattgt atctatgttag aatataatatg 780  
 tggatgtgc tatttataatggc tctttaatgt atgttcatgt accctcaaaa caaaatgtat 840  
 ttatgtgtt gtgaagtttc ctacttttattt ttctcataact ataacttggaa actaaaaaaa 900  
 aaaaaaaaaa aaaaaaa 916

<210> 16

<211> 205

<212> PRT

<213> Triticum aestivum

<400> 16

Pro Arg Val Glu Pro Asp Leu Ser Lys Leu Ala Leu Ala Gly His Ser  
 1 5 10 15

Arg Gly Gly Gln Thr Ala Phe Ala Val Ala Leu Gly Leu Gly Asp Ala  
 20 25 30

Lys Thr Lys Leu Glu Leu Lys Phe Ser Ala Leu Ile Gly Val Asp Pro  
 35 40 45

Val Ala Gly Val Ser Arg Ala Gln Gln Leu Glu Pro Lys Val Leu Thr  
 50 55 60

Phe Glu Pro Asp Cys Leu Asp Val Gly Met Pro Val Leu Val Met Gly  
65 70 75 80

Thr Gly Leu Gly Pro Lys His Ile Gly Gly Phe Pro Cys Ala Pro Val  
85 90 95

Gly Val Asn His Ala Glu Phe Tyr Lys Glu Cys Ala Pro Pro Arg Tyr  
100 105 110

His Leu Val Val Lys Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asn  
115 120 125

Val Pro Tyr Ile Ile Asn Asn Cys Met Cys Met Arg Asn Gln His Asp  
130 135 140

Thr Lys Asp Leu Ala Arg Arg Thr Met Gly Gly Ala Met Val Ala Phe  
145 150 155 160

Leu Arg Ala Lys Leu Arg Ile Asp Val Arg Asp Leu Ile Ala Ile Tyr  
165 170 175

His Asn Pro Glu Ile Ala Pro Ala Val Leu Asp Gln Val Asp Glu Phe  
180 185 190

Leu Pro Cys Phe Val Gly Arg Pro Asn Pro Ser Ser Val  
195 200 205

<210> 17

<211> 1242

<212> DNA

<213> Triticum aestivum

<400> 17

gcacgagggt aagacagaga gttggtgccc tgagctttag tgagagatgg cagcagcagc 60  
accccgagag acgatgaaca agtccggcgc cggcggcggag gttcccgagg cgttcacatc 120  
ggtgttccag ccgggaaagc ttgcgtcga ggcgatttag gtggatgaga atgcggcgcc 180  
gacaccaccc atccccgtgc tgatgtcgc acccaaggat gcaggaacct accccgtggc 240  
catgtcttg cacggcttct tcctccataa ccacttctac gaacaccttc tccggcacgt 300  
cgcatccac ggcttcatca ttgtcgcgccc coagttcagc atcagtatca taccttcggg 360  
tgacgcagag gacatgcgcg cggcagccaa ggtggcagac tggctccccc acggcctccc 420  
gtccgtctg cccaaaggcg tcgaggccga gctctcgaag ctgccttgg cggccacag 480  
ccgaggaggc cacacggctt tctccctggc cttggggcac gccaagaccc agctaacctt 540  
ctccgcgtc atcggactcg accccgtcgc cggcacgggg aagtccccc agetccagcc 600  
caagatcc tcacctacg cgtccctt cggcatggcg atgcccgtgc tggcatcg 660  
caccggcgtc ggcgaggaga agaagaacat attttccct ccctgcgcac ccaaggacgt 720  
gaaccacgccc gagttctacc gcgagtgcag gccccttcg tactacttt tgaccaagga 780  
ctacggcat ctggacatgc tggacacga ccccccaag ttcatcacct gcgtctgcaa 840  
ggatggaaac gggtgcagg gcaagatgc gaggtgcgtt gctggatca tggggcatt 900  
tcttaatgtc gccttgggt agaaagatgc agatctttag gccatactga gagacccggc 960  
ggttgcaccc accacgctt atccggttga gcaccgcgtg gcgtgaagca gcagctggct 1020  
catgccaatgg gttaccaggc cgcagggtgg tccactaaat aaagtacaga tttactgtac 1080  
ctctgagcaa tttgttccac ttgttccatgc aagctaaaac tgctgtcttgc ttacttctt 1140  
ctcctgcgag cctgcaatgg tacttctgtc ctgtttccag atgtatacta ctcctgttag 1200  
tataatctt ttttctgtgt ctaaaaaaaaaaaaaa aa 1242

<210> 18

<211> 319

<212> PRT

<213> Triticum aestivum

<400> 18  
 Met Ala Ala Ala Ala Pro Ala Glu Thr Met Asn Lys Ser Ala Ala Gly  
 1 5 10 15

Ala Glu Val Pro Glu Ala Phe Thr Ser Val Phe Gln Pro Gly Lys Leu  
 20 25 30

Ala Val Glu Ala Ile Gln Val Asp Glu Asn Ala Ala Pro Thr Pro Pro  
 35 40 45

Ile Pro Val Leu Ile Val Ala Pro Lys Asp Ala Gly Thr Tyr Pro Val  
 50 55 60

Ala Met Leu Leu His Gly Phe Phe Leu His Asn His Phe Tyr Glu His  
 65 70 75 80

Leu Leu Arg His Val Ala Ser His Gly Phe Ile Ile Val Ala Pro Gln  
 85 90 95

Phe Ser Ile Ser Ile Ile Pro Ser Gly Asp Ala Glu Asp Ile Ala Ala  
 100 105 110

Ala Ala Lys Val Ala Asp Trp Leu Pro Asp Gly Leu Pro Ser Val Leu  
 115 120 125

Pro Lys Gly Val Glu Pro Glu Leu Ser Lys Leu Ala Leu Ala Gly His  
 130 135 140

Ser Arg Gly Gly His Thr Ala Phe Ser Leu Ala Leu Gly His Ala Lys  
 145 150 155 160

Thr Gln Leu Thr Phe Ser Ala Leu Ile Gly Leu Asp Pro Val Ala Gly  
 165 170 175

Thr Gly Lys Ser Ser Gln Leu Gln Pro Lys Ile Leu Thr Tyr Glu Pro  
 180 185 190

Ser Ser Phe Gly Met Ala Met Pro Val Leu Val Ile Gly Thr Gly Leu  
 195 200 205

Gly Glu Glu Lys Lys Asn Ile Phe Phe Pro Pro Cys Ala Pro Lys Asp  
 210 215 220

Val Asn His Ala Glu Phe Tyr Arg Glu Cys Arg Pro Pro Cys Tyr Tyr  
 225 230 235 240

Phe Val Thr Lys Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asp Ala  
 245 250 255

Pro Lys Phe Ile Thr Cys Val Cys Lys Asp Gly Asn Gly Cys Lys Gly  
 260 265 270

Lys Met Arg Arg Cys Val Ala Gly Ile Met Val Ala Phe Leu Asn Ala  
 275 280 285

Ala Leu Gly Glu Lys Asp Ala Asp Leu Glu Ala Ile Leu Arg Asp Pro  
 290 295 300

Ala Val Ala Pro Thr Thr Leu Asp Pro Val Glu His Arg Val Ala  
 305 310 315

&lt;210&gt; 19

&lt;211&gt; 324

&lt;212&gt; PRT

<213> *Arabidopsis thaliana*

&lt;400&gt; 19

Met	Ala	Ala	Ile	Glu	Asp	Ser	Pro	Thr	Phe	Ser	Ser	Val	Val	Thr	Pro
1														10	15

Ala	Ala	Phe	Glu	Ile	Gly	Ser	Leu	Pro	Thr	Thr	Glu	Ile	Pro	Val	Asp
														20	30

Pro	Val	Glu	Asn	Asp	Ser	Thr	Ala	Pro	Pro	Lys	Pro	Val	Arg	Ile	Thr
														35	45

Cys	Pro	Thr	Val	Ala	Gly	Thr	Tyr	Pro	Val	Val	Leu	Phe	Phe	His	Gly
														50	60

Phe	Tyr	Leu	Arg	Asn	Tyr	Phe	Tyr	Ser	Asp	Val	Leu	Asn	His	Ile	Ala
														65	80

Ser	His	Gly	Tyr	Ile	Leu	Val	Ala	Pro	Gln	Leu	Cys	Lys	Leu	Leu	Pro
														85	95

Pro	Gly	Gly	Gln	Val	Glu	Val	Asp	Asp	Ala	Gly	Ser	Val	Ile	Asn	Trp
														100	110

Ala	Ser	Glu	Asn	Leu	Lys	Ala	His	Leu	Pro	Thr	Ser	Val	Asn	Ala	Asn
														115	125

Gly	Lys	Tyr	Thr	Ser	Leu	Val	Gly	His	Ser	Arg	Gly	Gly	Lys	Thr	Ala
														130	140

Phe	Ala	Val	Ala	Leu	Gly	His	Ala	Ala	Thr	Leu	Asp	Pro	Ser	Ile	Thr
														145	160

Phe	Ser	Ala	Leu	Ile	Gly	Ile	Asp	Pro	Val	Ala	Gly	Thr	Asn	Lys	Tyr
														165	175

Ile	Arg	Thr	Asp	Pro	His	Ile	Leu	Thr	Tyr	Lys	Pro	Glu	Ser	Phe	Glu
														180	190

Leu	Asp	Ile	Pro	Val	Ala	Val	Val	Gly	Thr	Gly	Leu	Gly	Pro	Lys	Trp
														195	205

Asn	Asn	Val	Met	Pro	Pro	Cys	Ala	Pro	Thr	Asp	Leu	Asn	His	Glu	Glu
														210	220

Phe	Tyr	Lys	Glu	Cys	Lys	Ala	Thr	Lys	Ala	His	'Phe	Val	Ala	Ala	Asp
														225	240

Tyr	Gly	His	Met	Asp	Met	Leu	Asp	Asp	Leu	Pro	Gly	Phe	Val	Gly	
														245	255

Phe	Met	Ala	Gly	Cys	Met	Cys	Lys	Asn	Gly	Gln	Arg	Lys	Lys	Ser	Glu
														260	270

Met	Arg	Ser	Phe	Val	Gly	Gly	Ile	Val	Val	Ala	Phe	Leu	Lys	Tyr	Ser
														275	285

Leu Trp Gly Glu Lys Ala Glu Ile Arg Leu Ile Val Lys Asp Pro Ser  
 290 295 300

Val Ser Pro Ala Lys Leu Asp Pro Ser Pro Glu Leu Glu Glu Ala Ser  
 305 310 315 320

Gly Ile Phe Val

<210> 20

<211> 318

<212> PRT

<213> *Arabidopsis thaliana*

<400> 20

Met Ser Ser Ser Ser Arg Asn Ala Phe Glu Asp Gly Lys Tyr Lys  
 1 5 10 15

Ser Asn Leu Leu Thr Leu Asp Ser Ser Arg Cys Cys Lys Ile Thr  
 20 25 30

Pro Ser Ser Arg Ala Ser Pro Ser Pro Pro Lys Gln Leu Leu Val Ala  
 35 40 45

Thr Pro Val Glu Glu Gly Asp Tyr Pro Val Val Met Leu Leu His Gly  
 50 55 60

Tyr Leu Leu Tyr Asn Ser Phe Tyr Ser Gln Leu Met Leu His Val Ser  
 65 70 75 80

Ser His Gly Phe Ile Leu Ile Ala Pro Gln Leu Tyr Ser Ile Ala Gly  
 85 90 95

Pro Asp Thr Met Asp Glu Ile Lys Ser Thr Ala Glu Ile Met Asp Trp  
 100 105 110

Leu Ser Val Gly Leu Asn His Phe Leu Pro Ala Gln Val Thr Pro Asn  
 115 120 125

Leu Ser Lys Phe Ala Leu Ser Gly His Ser Arg Gly Gly Lys Thr Ala  
 130 135 140

Phe Ala Val Ala Leu Lys Lys Phe Gly Tyr Ser Ser Asn Leu Lys Ile  
 145 150 155 160

Ser Thr Leu Ile Gly Ile Asp Pro Val Asp Gly Thr Gly Lys Gly Lys  
 165 170 175

Gln Thr Pro Pro Pro Val Leu Ala Tyr Leu Pro Asn Ser Phe Asp Leu  
 180 185 190

Asp Lys Thr Pro Ile Leu Val Ile Gly Ser Gly Leu Gly Glu Thr Ala  
 195 200 205

Arg Asn Pro Leu Phe Pro Pro Cys Ala Pro Pro Gly Val Asn His Arg  
 210 215 220

Glu Phe Phe Arg Glu Cys Gln Gly Pro Ala Trp His Phe Val Ala Lys  
 225 230 235 240

Asp Tyr Gly His Leu Asp Met Leu Asp Asp Asp Thr Lys Gly Ile Arg  
 245 250 255  
 Gly Lys Ser Ser Tyr Cys Leu Cys Lys Asn Gly Glu Glu Arg Arg Pro  
 260 265 270  
 Met Arg Arg Phe Val Gly Gly Leu Val Val Ser Phe Leu Lys Ala Tyr  
 275 280 285  
 Leu Glu Gly Asp Asp Arg Glu Leu Val Lys Ile Lys Asp Gly Cys His  
 290 295 300  
 Glu Asp Val Pro Val Glu Ile Gln Glu Phe Glu Val Ile Met  
 305 310 315  
 <210> 21  
 <211> 347  
 <212> PRT  
 <213> Chenopodium album  
 <400> 21  
 Met Ala Lys Leu Leu Leu Ile Phe Gly Val Phe Ile Phe Val Asn  
 1 5 10 15  
 Ser Gln Ala Gln Thr Phe Pro Thr Ile Leu Glu Lys His Asn Ser Glu  
 20 25 30  
 Lys Ile Thr Asp Val Phe His Lys Gly Asn Phe Gln Val Thr Asn Asn  
 35 40 45  
 Pro Ile Arg Val Lys Arg Tyr Glu Phe Ser Ala Pro Glu Pro Leu Ile  
 50 55 60  
 Ile Ile Ser Pro Lys Glu Ala Gly Val Tyr Pro Val Leu Leu Phe Ile  
 65 70 75 80  
 His Gly Thr Met Leu Ser Asn Glu Asp Tyr Ser Leu Phe Phe Asn Tyr  
 85 90 95  
 Ile Ala Ser His Gly Phe Ile Val Val Ala Pro Lys Leu Phe Arg Leu  
 100 105 110  
 Phe Pro Pro Lys Leu Pro Ser Gln Gln Asp Glu Ile Asp Met Ala Ala  
 115 120 125  
 Ser Val Ala Asn Trp Met Pro Leu Tyr Leu Gln Val Val Leu Gln Arg  
 130 135 140  
 Tyr Val Thr Gly Val Glu Gly Asp Leu Glu Lys Leu Ala Ile Ser Gly  
 145 150 155 160  
 His Ser Arg Gly Gly Lys Ser Ala Phe Ala Leu Ala Leu Gly Phe Ser  
 165 170 175  
 Asn Ile Lys Leu Asp Val Thr Phe Ser Ala Leu Ile Gly Val Asp Pro  
 180 185 190  
 Val Ala Gly Arg Ser Val Asp Asp Arg Thr Leu Pro His Val Leu Thr  
 195 200 205

Tyr Lys Pro Asn Ser Phe Asn Leu Ser Ile Pro Val Thr Val Ile Gly  
 210 215 220  
 Ser Gly Leu Gly Asn His Thr Ile Ser Cys Ala Pro Asn His Val Ser  
 225 230 235 240  
 His Gln Gln Phe Tyr Asp Glu Cys Lys Glu Asn Ser Ser His Phe Val  
 245 250 255  
 Ile Thr Lys Tyr Gly His Met Asp Met Leu Asn Glu Phe Arg Leu Ser  
 260 265 270  
 Pro Ile Ala Val Thr Met Ser Leu Met Cys Ala Gln Ser Phe Arg Pro  
 275 280 285  
 Lys Ala Thr Met Arg Arg Thr Leu Gly Gly Ile Met Val Ala Phe Leu  
 290 295 300  
 Asn Ala Tyr Phe Arg Asp Asp Gly Arg Gln Tyr Tyr Ala Ile Ile Ala  
 305 310 315 320  
 Asn Arg Ser Leu Ala Pro Thr Asn Leu Phe Ala Glu Lys Lys Gly Phe  
 325 330 335  
 Asn Phe Gly Phe Ala Thr Thr Tyr Ala Gln Leu  
 340 345

<210> 22  
 <211> 329  
 <212> PRT  
 <213> Citrus sinensis

<400> 22  
 Met Ala Ala Met Val Asp Ala Lys Pro Ala Ala Ser Val Gln Gly Thr  
 1 5 10 15  
 Pro Leu Leu Ala Thr Ala Thr Leu Pro Val Phe Thr Arg Gly Ile Tyr  
 20 25 30  
 Ser Thr Lys Arg Ile Thr Leu Glu Thr Ser Ser Pro Ser Ser Pro Pro  
 35 40 45  
 Pro Pro Lys Pro Leu Ile Ile Val Thr Pro Ala Gly Lys Gly Thr Phe  
 50 55 60  
 Asn Val Ile Leu Phe Leu His Gly Thr Ser Leu Ser Asn Lys Ser Tyr  
 65 70 75 80  
 Ser Lys Ile Phe Asp His Ile Ala Ser His Gly Phe Ile Val Val Ala  
 85 90 95  
 Pro Gln Leu Tyr Thr Ser Ile Pro Pro Pro Ser Ala Thr Asn Glu Leu  
 100 105 110  
 Asn Ser Ala Ala Glu Val Ala Glu Trp Leu Pro Gln Gly Leu Gln Gln  
 115 120 125

Asn Leu Pro Glu Asn Thr Glu Ala Asn Val Ser Leu Val Ala Val Met  
 130 135 140  
 Gly His Ser Arg Gly Gly Gln Thr Ala Phe Ala Leu Ser Leu Arg Tyr  
 145 150 155 160  
 Gly Phe Gly Ala Val Ile Gly Leu Asp Pro Val Ala Gly Thr Ser Lys  
 165 170 175  
 Thr Thr Gly Leu Asp Pro Ser Ile Leu Ser Phe Asp Ser Phe Asp Phe  
 180 185 190  
 Ser Ile Pro Val Thr Val Ile Gly Thr Gly Leu Gly Gly Val Ala Arg  
 195 200 205  
 Cys Ile Thr Ala Cys Ala Pro Glu Gly Ala Asn His Glu Glu Phe Phe  
 210 215 220  
 Asn Arg Cys Lys Asn Ser Ser Arg Ala His Phe Val Ala Thr Asp Tyr  
 225 230 235 240  
 Gly His Met Asp Ile Leu Asp Asp Asn Pro Ser Asp Val Lys Ser Trp  
 245 250 255  
 Ala Leu Ser Lys Tyr Phe Cys Lys Asn Gly Asn Glu Ser Arg Asp Pro  
 260 265 270  
 Met Arg Arg Cys Val Ser Gly Ile Val Val Ala Phe Leu Lys Asp Phe  
 275 280 285  
 Phe Tyr Gly Asp Ala Glu Asp Phe Arg Gln Ile Leu Lys Asp Pro Ser  
 290 295 300  
 Phe Ala Pro Ile Lys Leu Asp Ser Val Glu Tyr Ile Asp Ala Ser Ser  
 305 310 315 320  
 Met Leu Thr Thr His Val Lys Val  
 325

<210> 23  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:PCR primer P1

<400> 23  
 tttcatatgg cagcagcagc acccg

25

<210> 24  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:PCR primer P2

&lt;400&gt; 24

tttctcgagc tgcaatggta cttctgctc

29

&lt;210&gt; 25

&lt;211&gt; 1156

&lt;212&gt; DNA

&lt;213&gt; Triticum aestivum

&lt;400&gt; 25

gcacgaggcc atctccgccc	tgcttccgct	ctagcttcta	gctagctaag	taagatatgg	60
ctgcaatggc	gacgacggg	ttccaggcgg	gccaatgg	ggtggatgtg	120
acaagagtat	gatcccgaac	ctggccagc	cgttgatgg	ggtggccccc	180
gcgcgtacc	cgtcatecg	ttcctgcacg	gctggaaat	gctcaacagc	240
agctcctcac	acacgtcg	ccccatgg	tcatcgccg	cgcaccacag	300
tgggtccga	gccccatgc	gacgacatag	acgcccacaaa	aactggcttg	360
catatcatga	caaggggctc	gcccacgtcc	tcaaggacgt	gctcaaactt	420
agctgaccc	gtccaaagctg	gcccctagccg	gccatagccg	gagcatgtcg	480
ccgtcgccct	gggactaggg	gacgccaaga	ccaagctga	gctcaagttc	540
tcggcgtcg	ccccgtggcc	gggggttcca	gagcccaaca	ttggagccc	600
cttttgaacc	tgactgtctc	gacgtgggg	tgccgggt	ggtcatggg	660
gtcccaagca	catcgccg	tttccatgcg	ccccgtgg	cgtgaaccac	720
acaaggagt	cgccgcgc	cgctaccacc	tcgtggtca	ggactacggg	780
tgctggatga	caatgtgccc	tatatcatca	acaactgcat	gtgcatgagg	840
acaccaaaga	tcttgctagg	aggaccatgg	gaggagccat	ggtgccttc	900
aattgcgaat	cgatgttcgt	gatctcatcg	ccatatatca	ctcagggcta	960
ccgtcctgga	ccaagttgt	gagtttctc	cttgcttcgt	tggacggcca	1020
ctgtgtgaga	gttatattac	gtgcctatgc	ctatctat	ctcctaaata	1080
gtgcatggcc	ttgtcatggc	tcatgactac	tatagcacgt	ccagtcttcc	1140
aaaaaaaaaa	aaaaaa				1156

&lt;210&gt; 26

&lt;211&gt; 323

&lt;212&gt; PRT

&lt;213&gt; Triticum aestivum

&lt;400&gt; 26

Met	Ala	Ala	Met	Ala	Thr	Thr	Val	Phe	Gln	Ala	Gly	Pro	Met	Glu	Val
1															
														10	15

Asp	Val	Lys	His	Val	Asp	Lys	Ser	Met	Ile	Pro	Asn	Leu	Ala	Arg	Pro
														20	30

Leu	Met	Val	Val	Ala	Pro	Lys	Glu	Thr	Gly	Ala	Tyr	Pro	Val	Ile	Val
														35	45

Phe	Leu	His	Gly	Trp	Asn	Met	Leu	Asn	Ser	Trp	Tyr	Glu	Gln	Leu	Leu
														50	60

Thr	His	Val	Ala	Ser	His	Gly	Phe	Ile	Ala	Val	Ala	Pro	Gln	Leu	Tyr
														65	75

Trp	Met	Val	Ser	Glu	Pro	Asp	Ala	Asp	Asp	Ile	Asp	Ala	Thr	Lys	Arg
														85	95

Ile	Thr	Asn	Trp	Leu	Ala	Asp	His	Asp	Lys	Gly	Leu	Ala	His	Val	Leu
														100	110

Lys Asp Val Leu Lys Leu Glu His Val Glu Pro Asp Leu Ser Lys Leu  
 115 120 125  
 Ala Leu Ala Gly His Ser Arg Gly Gly Gln Thr Ala Phe Ala Val Ala  
 130 135 140  
 Leu Gly Leu Gly Asp Ala Lys Thr Lys Leu Glu Leu Lys Phe Ser Ala  
 145 150 155 160  
 Leu Ile Gly Val Asp Pro Val Ala Gly Val Ser Arg Ala Gln Gln Leu  
 165 170 175  
 Glu Pro Lys Val Leu Thr Phe Glu Pro Asp Cys Leu Asp Val Gly Met  
 180 185 190  
 Pro Val Leu Val Met Gly Thr Gly Leu Gly Pro Lys His Ile Gly Gly  
 195 200 205  
 Phe Pro Cys Ala Pro Val Gly Val Asn His Ala Glu Phe Tyr Lys Glu  
 210 215 220  
 Cys Ala Pro Pro Arg Tyr His Leu Val Val Lys Asp Tyr Gly His Leu  
 225 230 235 240  
 Asp Met Leu Asp Asp Asn Val Pro Tyr Ile Ile Asn Asn Cys Met Cys  
 245 250 255  
 Met Arg Asn Gln His Asp Thr Lys Asp Leu Ala Arg Arg Thr Met Gly  
 260 265 270  
 Gly Ala Met Val Ala Phe Leu Arg Ala Lys Leu Arg Ile Asp Val Arg  
 275 280 285  
 Asp Leu Ile Ala Ile Tyr His Asn Pro Glu Ile Ala Pro Ala Val Leu  
 290 295 300  
 Asp Gln Val Asp Glu Phe Leu Pro Cys Phe Val Gly Arg Pro Asn Pro  
 305 310 315 320  
 Ser Ser Val

<210> 27  
 <211> 1274  
 <212> DNA  
 <213> Zea mays

<400> 27  
 ccacgcgtcc gcccgtactc acgcccgtact gtctgatcgg caacacccctcg tgtacacgt 60  
 ggcccccggaga tatggcgcca tcgccccgtgg ccatcgccac ggccgtgttc cagcgggggc 120  
 cactccgcgt ggaggcgagg cacgtcgact acagccagggt ccccaagccgtg cccaaagccgc 180  
 tcatgggtgg cggcccccaccc gacgcggcgct tcctacccgtt ggccgtcttc ctgcacggct 240  
 gcaaacacgggt caacagctgg tatgagagcc tcctgtcgca cgtcgcgtcc cacgggttca 300  
 tcgcccgtggc gccccagctc tactgcgtga cgtcaacat gaacgactt aaggacatcg 360  
 acgcaccacgg gcaagggttccgc gcctggctcg cccacaagca gcaaggctcg ggcacgtgc 420  
 tcgccaacat cttccagctc cacggcgtga gggccggaccc ttcggatgt ggcgtggccg 480  
 gcccacccgg cggccggcgac acggccctcg ccgtggactt cggggctcgga ccccccgcct 540  
 cggacgacgca cgacaacaac gcagacgca gacatcgcc ggcggcggtt ccactcaagt 600  
 tctcccgctt gatcgccgtg gaccccggtgg cggggcttac caagcaggcg cagggtggagc 660  
 cgaagggtgtt gaccccggtgg ccccggtttcc tcgacccggg gatgcggcgcttggcg 720

gcacggggct cggcccaag cacgtggcg ggccgcgtg cgcccccgq ggcgtcaacc 780  
 acggccgagtt ctacgacgag tgcgcgcgc cgcggatcca cgtcgctg aggactacg 840  
 ggcacatgga catgctggac gacgacggcg tgccctacgt catcaacaac tgcatgtgca 900  
 tgaggaacac caaggacacc aaggacctcg ccaggagggc catcgggga gccgtgggtg 960  
 cgttcctcag ggccacgctg gaggacgacg acgaggatct caaggtcg 1020  
 gccctggcct ctcgcgcgc gtgctggacc cagttggca tgacttggct tgaatagcaa 1080  
 ggctggcgga gtgtgctaca ttgtcgctcc ttatttacac acacatacca catgtttctt 1140  
 ttccattttgc tcctcgatcg tgcatgtcc cacttgccac aactgtccag tccgctgggtg 1200  
 tccaaatgaat agcaaggatg tgaccctaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1260  
 aaaaaaaaaa aaag 1274

&lt;210&gt; 28

&lt;211&gt; 333

&lt;212&gt; PRT

&lt;213&gt; Zea mays

&lt;400&gt; 28

Met	Ala	Ala	Ser	Pro	Val	Ala	Ile	Gly	Thr	Ala	Val	Phe	Gln	Arg	Gly
1					5				10				15		

Pro	Leu	Arg	Val	Glu	Ala	Arg	His	Val	Asp	Tyr	Ser	Gln	Val	Pro	Ser
					20			25				30			

Val	Pro	Lys	Pro	Leu	Met	Val	Val	Ala	Pro	Thr	Asp	Ala	Gly	Val	Tyr
					35			40				45			

Pro	Val	Ala	Val	Phe	Leu	His	Gly	Cys	Asn	Thr	Val	Asn	Ser	Trp	Tyr
					50			55			60				

Glu	Ser	Leu	Leu	Ser	His	Val	Ala	Ser	His	Gly	Phe	Ile	Ala	Val	Ala
					65			70			75			80	

Pro	Gln	Leu	Tyr	Cys	Val	Thr	Leu	Asn	Met	Asn	Asp	Leu	Lys	Asp	Ile
					85			90				95			

Asp	Ala	Thr	Arg	Gln	Val	Thr	Ala	Trp	Leu	Ala	Asp	Lys	Gln	Gln	Gly
					100			105			110				

Leu	Ala	His	Val	Leu	Ala	Asn	Ile	Leu	Gln	Leu	His	Gly	Val	Arg	Pro
					115			120			125				

Asp	Leu	Ser	Arg	Leu	Ala	Leu	Ala	Gly	His	Ser	Arg	Gly	Gly	Asp	Thr
					130			135			140				

Ala	Phe	Ala	Val	Ala	Leu	Gly	Leu	Gly	Pro	Ala	Ala	Ser	Asp	Asp	Asp
					145			150			155			160	

Asp	Asn	Asn	Ala	Asp	Ala	Gly	Thr	Ser	Pro	Ala	Ala	Leu	Pro	Leu	Lys
					165			170			175				

Phe	Ser	Ala	Leu	Ile	Gly	Val	Asp	Pro	Val	Ala	Gly	Leu	Ser	Lys	Gln
					180			185			190				

Ala	Gln	Val	Glu	Pro	Lys	Val	Leu	Thr	Phe	Arg	Pro	Arg	Ser	Leu	Asp
					195			200			205				

Pro	Gly	Met	Pro	Ala	Leu	Val	Val	Gly	Thr	Gly	Leu	Gly	Pro	Lys	His
					210			215			220				

Val Gly Gly Pro Pro Cys Ala Pro Ala Gly Val Asn His Ala Glu Phe  
225 230 235 240

Tyr Asp Glu Cys Ala Pro Pro Arg Tyr His Val Val Leu Arg Asp Tyr  
245 250 255

Gly His Met Asp Met Leu Asp Asp Asp Gly Val Pro Tyr Val Ile Asn  
260 265 270

Asn Cys Met Cys Met Arg Asn Thr Lys Asp Thr Lys Asp Leu Ala Arg  
275 280 285

Arg Ala Ile Gly Gly Ala Val Val Ala Phe Leu Arg Ala Thr Leu Glu  
290 295 300

Asp Asp Asp Glu Asp Leu Lys Val Val Leu Glu Asn Arg Pro Gly Leu  
305 310 315 320

Ser Pro Ala Val Leu Asp Pro Val Gly His Asp Leu Ala  
325 330

<210> 29

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P3

<400> 29

ttccatggcg gcatgcgggg tggc

24

<210> 30

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P4

<400> 30

ttgcggccgc cagccttgct attcaagc

28